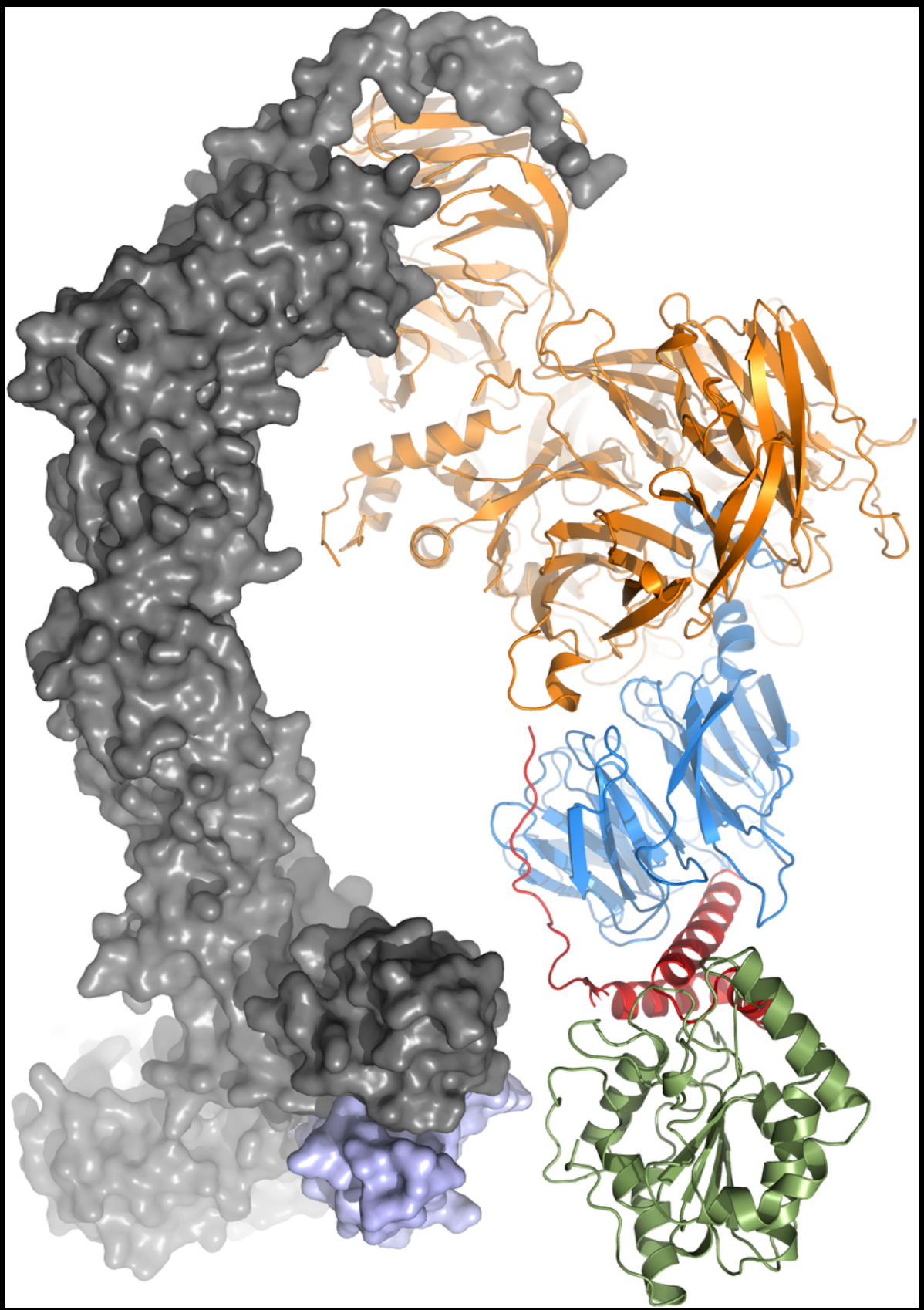
Structural Biology Related to HIV/AIDS - 2016



Thursday, June 23 – Friday, June 24, 2016
Ruth L. Kirschstein Auditorium

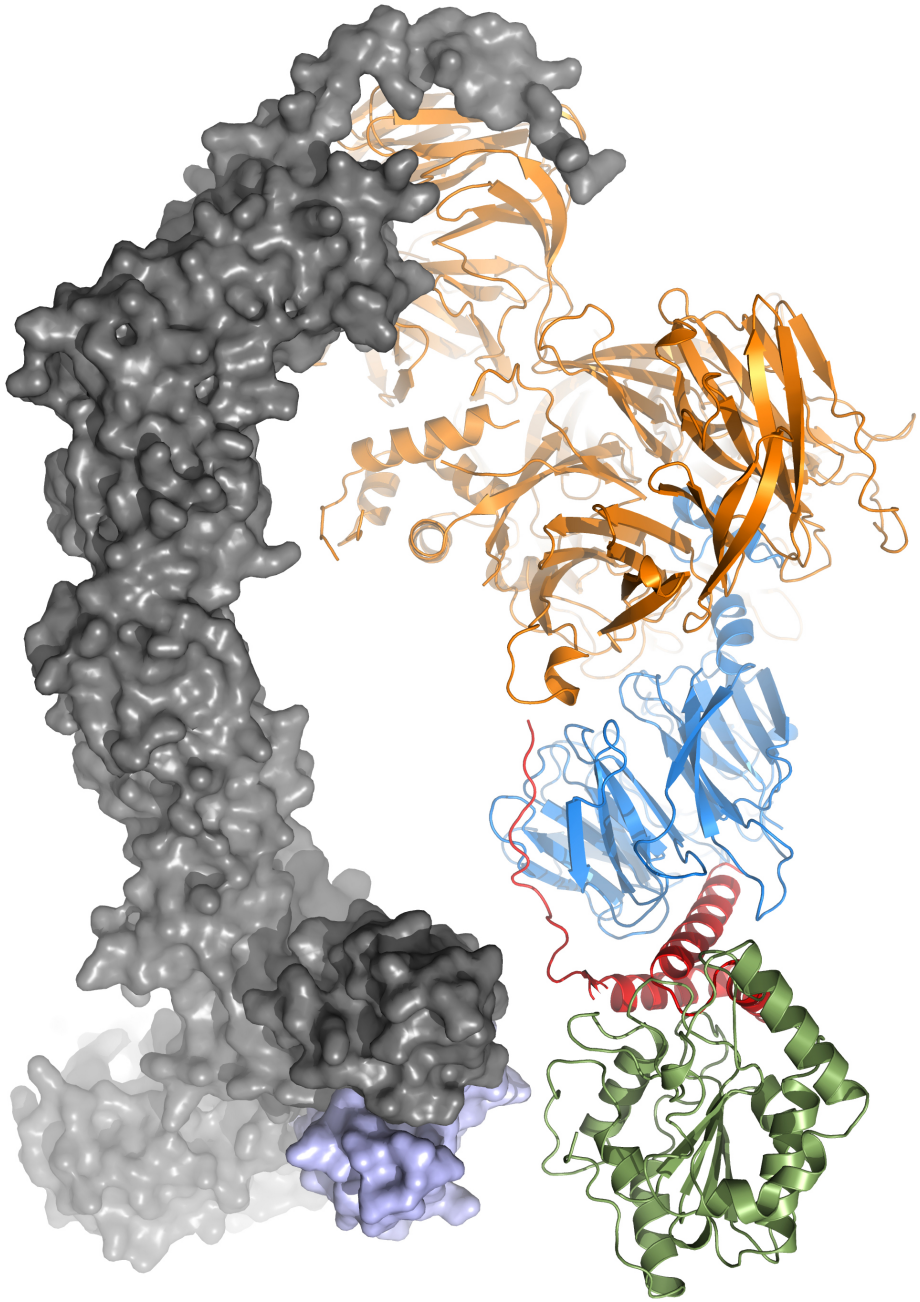
Natcher Conference Center
Bethesda, Maryland

NIH National Institute of General Medical Sciences

NIH National Institute of Allergy and Infectious Diseases

About the Cover:

X-ray crystal structure of HIV-1 Vpr (red) in complex with uracil DNA glycosylase 2 (UNG2, green) and the Cul4-DCAF1 E3 ligase, comprising DDB1- and CUL4-associated factor 1 (DCAF1, blue), damage-specific DNA binding protein 1 (DDB1, orange), Cullin4 (CUL4, gray) and RING H2 finger protein (RBX1, purple). Image courtesy of Ying Wu, Guillermo Calero, Jinwoo Ahn, and Angela Gronenborn of the Pittsburgh Center for HIV-1 Protein Interactions.



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Agenda - Structural Biology Related to HIV/AIDS – 2016

DAY 1

8:00 – 8:15 **Carl Dieffenbach** (National Institute of Allergy and Infectious Diseases)
Opening Remarks

Session I: Reports from Specialized Centers (P50); Session Chair: Wes Sundquist

8:15 – 9:15 The Center for HIV RNA Studies (CRNA)

Michael Summers (University of Maryland Baltimore County)
Influence of 5'-start Site Heterogeneity and Capping on HIV-1 RNA Structure and Fate

Victoria D'Souza (Harvard University)
Understanding Translational Regulation in HIV-1

Owen Pornillos (University of Virginia)
Crystal Structure of the CA-SP1 Assembly and Maturation Switch

Sanford Simon (Rockefeller University)
Steps in the Assembly of HIV-1

9:15 – 10:15 The Center for HIV Accessory and Regulatory Complexes (HARC)

Nevan Krogan (University of California San Francisco)
The HARC Center: Progress and Overview

Alex Marson (University of California San Francisco)
Genome Engineering Primary Human T Cells to Test Function of Host Factors in HIV Pathogenesis

Alan Frankel (University of California San Francisco)
Functional Segregation of Overlapping Genes in HIV

Charles Craik (University of California San Francisco)
Identifying Recombinant Antibodies to Challenging HIV Related Targets

10:15 – 10:45 BREAK

Session II: Reports from Specialized Centers (P50) continued; Session Chair: Irwin Chaiken

10:45 – 11:45 The HIV Interaction and Viral Evolution Center (HIVE)

Arthur Olson (The Scripps Research Institute)
HIVE Center: HIV Interactions and Viral Evolution of Drug Resistance

Mamuka Kvaratskhelia (Ohio State University)
HIV-1 Integrase Binds the Viral RNA Genome and Is Essential During Virion Morphogenesis

Eddie Arnold (Rutgers University)
A New Class of Allosteric HIV-1 Integrase Inhibitors Identified by Crystallographic Fragment Screening of the Catalytic Core Domain

Crystal Structure of Prototype Foamy Virus PR-RT Polyprotein Shows Unique Architecture Among Retroviruses: Implications for Function

Stefan Sarafianos (University of Missouri, Columbia)
Visualization of Viral RNA and DNA Dynamics During HIV infection

11:45 – 1:00 LUNCH

1:00 – 3:00 POSTER SESSION

Session III: Reports from Specialized Centers (P50) continued; Session Chair: Alan Frankel

3:00 – 4:00 The Pittsburgh Center for HIV Protein Interactions (PCHPI)

Tatyana Polenova (University of Delaware)
Dynamic Regulation of HIV-1 Capsid Assembly, Maturation, and Interactions with Host Factors by Integrated MAS NMR and MD Simulations

Jacek Skowronski (Case Western Reserve University)
HIV-1 and HIV-2 Exhibit Divergent Interactions with DNA Repair Enzymes

Guillermo Calero (University of Pittsburgh)
To Repair or Not Repair: The X-Ray Structure of the DDB1-DCAF1-Vpr-UNG2 Complex

4:00 – 5:00 The Center for the Structural Biology of Cellular Host Elements in Egress, Trafficking, and Assembly of HIV (CHEETAH)

Wes Sundquist (University of Utah)
Introduction to the Center

Michael Kay (University of Utah)
D-Peptide Fusion Inhibitor Protects Against High-Dose SHIV Challenge

Tom Hope (Northwestern University)
Visualizing HIV Uncoating in Living Cells

Barbie Ganser-Pornillos (University of Virginia)
Structural Studies of TRIM5α

5:00 ADJOURN FOR DAY

DAY TWO

Session IV: Invited Speakers, Session Chair: Elizabeth Church

8:00 – 8:30 **Debora Marks** (Harvard Medical School)
Structure and Function of RNA and Proteins from Natural Sequence Variation

8:30 – 9:00 **Kushol Gupta** (University of Pennsylvannia)
Structural Basis for Inhibitor-Induced Aggregation of HIV Integrase

Session V: Selected Posters, Session Chair: Alice Telesnitsky

9:00 – 9:30 **Lars-Anders Carlson** (University of California Berkeley)
Biochemical Reconstitution of Selective HIV-1 Genome Packaging

9:30 – 10:00 **Pamela Bjorkman** (California Institute of Technology)
Structure of a Natively-Glycosylated HIV-1 Env Reveals a New Mode for VH1-2 Antibody Recognition of the CD4 Binding Site Relevant to Vaccine Design

10:00 – 10:30 BREAK

Session V: Targeting Envelope, Session Chair: Arthur Olson

10:30 – 11:15 P01: Structure-Based Antagonism of HIV-1 Envelope Function in Cell Entry

Irwin Chaiken (Drexel University)
Overview of the Program and Recent Progress

Ernesto Freire (Johns Hopkins University)
Conformational Transformations Triggered by Different Inhibitor Classes

Alon Herschhorn (Dana-Farber Cancer Institute)
An Intermediate State of the HIV-1 Envelope Glycoproteins on the Entry Pathway

Session VI: Protease Drug Resistance, Angela Gronenborn:

11:15 – 12:00 P01: The Interdependency of Drug Resistance Evolution and Drug Design: HIV-1 Protease

Celia Schiffer (UMass Medical School)
Project Overview

Rieko Ishima (University of Pittsburgh)
Establish a Strategy to Elucidate Molecular Mechanisms Leading to Drug Resistance

Daniel Bolon (UMass Medical School)
Systematic Exploration of Mutational Pathways to Drug Resistance in HIV Protease

12:00 – 1:00 LUNCH
(SAB Lunch, Room B; Please bring your lunch.)

1:00 – 3:00 POSTER SESSION
(1:00 – 3:00 NIGMS Centers Scientific Review Board; Room B)

Session VIII: Selected Posters Cont., Session Chair: Celia Schiffer

3:00 – 3:30 **Ursula Schulze-Gahmen** (University of California Berkeley)
Insights into HIV-1 Proviral Transcription from an Integrative Structure of the Tat:AFF4:P-TEFb:TAR Complex

3:30 – 4:00 **Guney Boso** (Sanford Burnham Institute)
Arrayed Analysis of Immune Evasion: High Content Imaging Screen Reveals Novel Targets of HIV-1 Vpu

4:00 – 4:30 **Gregory Melikyan** (Emory University)
Real-Time Imaging of Single HIV-1 Uncoating in Cells

4:30 – 5:00 **Markus Thali** (University of Vermont)
More is Less: Enhancing Virus-Induced Membrane Fusion to Inhibit Viral Spread

5:00 ADJOURN MEETING

Mark Your Calendars!

Structural Biology Related to HIV/AIDS – 2017
Thursday June 29 – Friday June 30, 2017
Natcher Conference Center, Bethesda, Maryland

About the Poster Sessions

Thursday	**1:00 – 3:00**	**T-numbered posters**
Friday	**1:00 – 3:00**	**F-numbered posters**

Posters may be put up the morning of your session and left all day. Please remember to remove your poster from the board at the end of the assigned day. There are too many for posters to be left up for the whole meeting.

TOC

Thursday Posters

TOC

Computational Methods and Modeling

T1. Identifying and Incorporating Water-Mediated Interactions in Drug Discovery
Jiaye Guo,[1] and Robert C. Rizzo[2,3,4]
[1]Graduate Program in Biochemistry and Structural Biology, Stony Brook University, Stony Brook, New York 11794; [2]Department of Applied Mathematics & Statistics, Stony Brook University, Stony Brook, New York 11794; [3]Institute of Chemical Biology & Drug Discovery, Stony Brook University, Stony Brook, New York 11794; [4]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, New York 11794

T2. A Genetic Algorithm for DOCK to Aid in *De Novo* Design
Courtney D. Singleton[1], Lauren E. Prentis[2], and Robert C. Rizzo[3,4,5]
[1]Department of Pharmacology, [2]Department of Biochemistry & Structural Biology, [3]Department of Applied Mathematics & Statistics, [4]Institute of Chemical Biology & Drug Discovery, [5]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

T3. FightAIDS@Home Phase II: Refinement of Massive HIV Virtual Screening Experiments Using Large-Scale Molecular Dynamics Simulations
William F. Flynn[1,2], Junchao Xia[2], Nanjie Deng[2], Stefano Forli[3], Arthur Olson[3], and Ronald M. Levy[2]
[1]Department of Physics and Astronomy, Rutgers University, Piscataway, NJ; [2]Center for Biophysics and Computational Biology, Department of Chemistry, Temple University, Philadelphia, PA; [3]Molecular Graphics Laboratory, Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA

T4. Animating the Science of HIV
Janet Iwasa
Department of Biochemistry, University of Utah

T5. Coarse-grained (CG) Computer Models of Key Stages in the HIV-1 Lifecycle
J. M. A. Grime and G. A. Voth
[1]Department of Chemistry, Institute for Biophysical Dynamics, James Franck Institute, and Computation Institute, The University of Chicago, Chicago, Illinois 60637, USA

TOC

Antibody – Envelope Interactions and Virus Spread

T6. Modeling Affinity Maturation of Anti-HIV Antibodies Targeting gp120
Dzmitry Padhorny and Dima Kozakov
Laufer Center for Physics and Structural Biology, Stony Brook University

T7. The Broadest bnAbs Target More Conserved HIV-1 Env Epitopes
Hongjun Bai, Merlin L. Robb, Nelson L. Michael and Morgane Rolland
US Military HIV Research Program, Silver Spring, Maryland 20910, USA

T8. Structural Analysis of an HIV-1 Broadly Neutralizing V3-Glycan B-cell Lineage

Fera D[1], Bonsigniori M[2], Kreider E[3],Meyerhoff R[2], Bradley T[2], Wiehe K[2], Alam SM[2], Hwang KK[2], Saunders KO[2], Zhang R[2], Gladden MA[2], Monroe A[2], Kumar A[2], Xia SM[2], Cooper M[2], Jette CA[1], Pier BW[1], Montefiori DC, Trama A, Liao HX[2], Kepler TB[4], Gao F[2], Shaw GM[3], Hahn B[3], Moody MA[2], Gao F[2], Mascola JR, Haynes BF[2], and Harrison SC[1,5]

[1]Laboratory of Molecular Medicine and [5]HHMI, Boston Children's Hospital, Harvard Medical School, Boston, MA; [2]Department of Medicine, Duke University School of Medicine, Duke University Medical Center, and Duke Human Vaccine Institute, Durham, NC; [3]Departments of Medicine and Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [4]Departments of Microbiology, and Mathematics and Statistics, Boston University, Boston, MA; [5]Vaccine Research Center, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

T9. A Protective Role for CD169 in Limiting Systemic Spread of a Pathogenic Retrovirus

Pradeep D. Uchil[1], Ruoxi Pi[1], John D. Ventura[1], Kelsey A. Haugh[1], Xaver Sewald[2], and Walther Mothes[1*]

*[1]Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06510; [2]Max von Pettenkofer-Institute, Ludwig-Maximilians-University Munich, Munich, Germany; *corresponding author*

T10. Functional Interplay Between Murine Leukemia Virus Glycogag, Surface Glycoprotein and Serinc5 Modulates Virus Entry

Yadvinder S Ahi[1], Shu Zhang[1], Delphine Muriaux[2], Amin Feizpour[3], Björn M Reinhard[3], Rahm Gummuluru[3], and Alan Rein[1]

[1]NCI, HIV DRP, Frederick, MD; [2]CNRS, Membrane Domains and Viral Assembly, Montpellier, France; [3]Boston University, Boston, MA

T11. Multi-Dimensional Profiling of Primary Human CD4+ T Memory Cells

Lara Manganaro[1], Jeffrey R. Johnson[2,3,4], Patrick Hong[1], Benhur Lee[1], Nevan Krogan[2,3,4] and Viviana Simon[1,5,6]

[1]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [2]Gladstone Institute of Virology and Immunology, 1650 Owens Street, San Francisco, CA 94158, USA; [3]University of California, San Francisco, CA 94158, USA; [4]QB3, California Institute for Quantitative Biosciences, San Francisco, CA 94158, USA; [5]Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA; [6]Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, New York, USA

TOC

Envelope Structure

T12. Understanding Glycan Type Specificity in Highly Glycosylated Proteins

Artem Krantsevich and David F. Green

Stony Brook University

T13. Insights into Structures and Dynamics of Variable Regions of Major Subtypes of HIV-1 Gp120

Tuoling Qiu[1], and David F. Green[1, 2]

[1]Chemistry Department, Stony Brook University, Stony Brook, NY 11794; [2]Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY 11794

T14. Mapping the Conformational Space of Glycoconjugate-Linked Carbohydrates

Xindi Li[1] and David F. Green[1, 2, 3, 4]

[1]Department of Applied Mathematics & Statistics, [2]Department of Chemistry, [3]Laufer Center for Physical and Quantitative Biology, [4]Graduate Program in Biochemistry and Structural Biology, Stony Brook University, Stony Brook, New York 11794-3600, United States

T15. Deciphering the Mechanisms of HIV-1 Entry Using Novel Env Heterotrimers

Mukta D. Khasnis and Michael Root

Department of Biochemistry and Molecular Biology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA

T16. Structural Dynamics of HIV Env Glycoproteins: A Link Between Structural, Functional, and Phenotypic Variation Among Isolates

Kelly K. Lee[1], Hans Verkerke[1], Miklos Guttman[1], James A. Williams[1], Yu Liang[1], Thaddeus M. Davenport[1], Cassie Simonich[1,2], Shiu-Lok Hu[1], and Julie Overbaugh[1,2]

[1]University of Washington, [2]Fred Hutchinson Cancer Research Center, Seattle, WA

T17. On the Role of the V3 Loop in the Conformational Thermodynamics of Bridging Sheet Formation in HIV-1 Gp120: On-the-fly Parameterization Free-energy Calculations of the SOSIP BG505 Protomer

Alexis Paz[1], Matthew Cameron[1], and Cameron F. Abrams[1,2]

[1]Department of Chemical and Biological Engineering, Drexel University, 3141 Chestnut St., 19104, Philadelphia, PA (US); [2]Department of Biochemistry and Molecular Biology, Drexel College of Medicine, 25 North 15th Street, Philadelphia, PA (US)

T18. Single-Molecule FRET Delineates Asymmetric Trimer Conformations During HIV-1 Entry

Xiaochu Ma[1], Alon Herschhorn[2,3], John D. Ventura[1], Daniel S. Terry[4], Jason Gorman[5], Jonathan R. Grover[1], Xinyu Hong[1], Zhou Zhou[4], Hong Zhao[4], Roger B. Altman[4], James Arthos[6], Peter D. Kwong[5], Joseph Sodroski[2,3,7], Scott C. Blanchard[4], Walther Mothes[1*], and James B. Munro[8*]

*[1]Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06536, USA; [2]Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, Boston, MA 02215, USA; [3]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA 02115, USA; [4]Department of Physiology and Biophysics, Weill Cornell Medical College of Cornell University, New York, NY 10021, USA; [5]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA; [6]Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA; [7]Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health, Boston, MA 02115, USA; [8]Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111, USA; *Correspondence to: Walther Mothes (walther.mothes@yale.edu) and James Munro (James.Munro@tufts.edu)*

T19. Investigating Conformational Transitions in HIV-1 Env Using Combinations of CD4 Antagonists, Chemokine Receptor Antagonists and Fusion Inhibitors
Koree W. Ahn and Michael J. Root
Thomas Jefferson University, United States of America

TOC

Targeting Envelope

T20. Cyclic Peptide Triazole Rigid-Receptor Docking and Molecular Dynamics Simulation in Three Different Gp120 States: Comparison of Targets for Future HIV-1 Antagonist Optimization
Francesca Moraca[1], Adel A. Rashad[2], Kriti Acharya[2], Irwin Chaiken[2] and Cameron F. Abrams[1]
[1]Department of Chemical and Biological Engineering, Drexel University, 3141 Chestnut St., 19104, Philadelphia, PA (US); [2]Department of Biochemistry and Molecular Biology, Drexel College of Medicine, 25 North 15th Street, Philadelphia, PA (US)

T21. Generation and Characterization of HIV-1 Escape Mutants to Peptide Triazole Entry Inhibitors
Andrew P. Holmes[1,2], Adel Ahmed[2], Lauren D. Bailey[2], Katie Kercher[1], William Dampier[1], Michael Nonnemacher[1], Michael Root[3], and Irwin Chaiken[2]
[1]Department of Microbiology and Immunology, Drexel University College of Medicine; [2]Department of Biochemistry and Molecular Biology, Drexel University College of Medicine; [3]Department of Biochemistry and Molecular Biology, Thomas Jefferson University

T22. Optimization of Macrocyclic Peptide Triazole HIV-1 Inactivators
Adel Ahmed Rashad*, Kriti Acharya*, Rachna Aneja, Ann Haftl and Irwin Chaiken
*Department of Biochemistry & Molecular Biology, Drexel University College of Medicine, Philadelphia, Pennsylvania 19102; *Corresponding authors: adel.ahmed@drexelmed.edu, ka536@drexel.edu*

T23. Structure-Based Optimization of Small-Molecule CD4-Mimics: Inhibitors of HIV-1 Entry
Sharon M. Kirk, Melissa C. Grenier, Althea E. Gaffney, Bruno Melillo, Amos B. Smith, III
Department of Chemistry, University of Pennsylvania

T24. DeNovo Design and Refinement of Inhibitors Targeting HIV Entry
Brian C. Fochtman[1] William J. Allen[2] and Robert C. Rizzo[2,3,4]
[1]Department of Biochemistry and Cellular Biology, [2]Department of Applied Mathematics & Statistics, [3]Institute of Chemical Biology & Drug Discovery, [4]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

T25. HIV-1 Lytic Inactivation by Dual Acting Virus Entry Inhibitor Occurs Through Combined Interactions with gp120 and gp41 Subunits of Virus Env Protein Trimer
Bibek Parajuli[1], Kriti Acharya[1], Reina Yu[1], Adel A. Rashad[1], Cameron F. Abrams[2], Irwin M. Chaiken[1*]
[1]Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, Pennsylvania 19102, United States; [2]Department of Chemical and Biological Engineering, Drexel University, Philadelphia, Pennsylvania 19104, United States

T26. Design, Synthesis, and Biological Evaluation of Helical Spiroligomers Targeting HIV-1 Gp41

Cheong, J. E. and Schafmeister, C. E.
Department of Chemistry, Temple University

T27. Molecular Dynamics Simulations of HIVgp41 Reveal Energetically Favorable Interfaces for Small-Molecule Inhibitors

T. Dwight McGee Jr.[1], and Robert Rizzo[1,2,3]
[1]Department of Applied Mathematics & Statistics, [2]Institute of Chemical Biology & Drug Discovery, [3]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

T28. Expression of HERV-K108 Envelope Interferes with HIV-1 Production

Sandra Terry[1], Alvaro Cuesta-Dominguez[1], Lara Manganaro[1], Daria Brinzevich[1], Viviana Simon[1,2], and Lubbertus C.F. Mulder[1, 2]
[1]Department of Microbiology, [2]Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY

Uncoating and TRIM5α

TOC

T29. Minor Sequence Differences in HIV-1_{NL4-3} and HIV-1_{LAI} Capsid Cause Distinct Capsid Uncoating and Host Cell Infectivity Phenotypes

Douglas K. Fischer[1,2,4], Simon C. Watkins[3,4], Masahiro Yamashita[4,5], Tatyana Polenova[4,6], and Zandrea Ambrose[1,2,4]
[1]Molecular Virology and Microbiology Program, [2]Division of Infectious Diseases, Department of Medicine, [3]Department of Cell Biology and Physiology, and [4]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA; [5]Aaron Diamond AIDS Research Center, New York, NY; and [6]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE

T30. Real-Time Imaging of Single HIV-1 Uncoating in Cells

Ashwanth C. Francis[1], Mariana Marin[1], Jiong Shi[2], Christopher Aiken[2] and Gregory B. Melikyan[1,3]
[1]Department of Pediatric, Emory University School of Medicine, Atlanta, GA; [2]Department of Pathology, Microbiology and Immunology, Vanderbilt University, Nashville, TN; [3]Children's Healthcare of Atlanta, Atlanta, GA

T31. Correlation of Infectivity and Imaged Individual HIV Particle Behavior Validates the Early Uncoating Model During HIV Infection

João I. Mamede, Gianguido C. Cianci, Meegan Anderson, Thomas J Hope
Northwestern University Feinberg School of Medicine, Department of Cell and Molecular Biology, Chicago, IL

T32. Development of Cryo-CLEM Methods to Elucidate the Intracellular Structure of TRIM5α Bodies

Stephen D. Carter[1], Shrawan K. Mageswaran[1], Joao I. Mamede[3], Tom J. Hope[3], Joachim Frank[4,5], Zachary Freyberg[6,7], and Grant J. Jensen[1,2]

[1]Division of Biology and [2]Howard Hughes Medical Institute (HHMI), California Institute of Technology, Pasadena, CA 91125, USA; [3]Department of Cell and Molecular Biology, Northwestern University, Chicago, IL 60611-3008; [4]HHMI; Department of Biochemistry and Molecular Biophysics, Columbia University, New York, New York 10032, USA; [5]Department of Biological Sciences, Columbia University, New York, New York 10027, USA; [6]Department of Psychiatry, College of Physicians & Surgeons, Columbia University, New York, New York 10032, USA; [7]Division of Molecular Therapeutics, New York State Psychiatric Institute, New York, New York 10032, USA

T33. Characterization of TRIM5 Assembly and Activation Using Chimeric "MiniTRIMs"

Jonathan M. Wagner[1], Marcin D. Roganowicz[1], Katarzyna Skorupka[1], Steven L. Alam[2], Devin Christensen[2], Ginna Doss[1], Yueping Wan[1], Gabriel A. Frank[3], Barbie K. Ganser-Pornillos[1], Wesley I. Sundquist[2], and Owen Pornillos[1]

[1]Department of Molecular Physiology and Biological Physics, [2]Department of Biochemistry, University of Utah, Salt Lake City, UT 84112, U.S.A.; [3]Faculty of Biology, Technion - Israel Institute of Technology, Haifa 320003, Israel

T34. Analysis of TRIM5α SPRY Domain Packing Against Its Coiled-Coil Domain

Marcin D. Roganowicz[1], Santanu Mukherjee[2], Katarzyna Skorupka[1], Damian Dawidowski[3], David S. Cafiso[3], Edward M. Campbell[2], Owen Pornillos[1]

[1]Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA; [2]Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, Maywood, IL; [3]Department of Chemistry, University of Virginia, Charlottesville, VA

T35. Dynamic Allostery in HIV-1 Capsid Interactions with Restriction Factor TRIM5 Revealed by Magic Angle Spinning NMR

Caitlin M Quinn[1,2], Mingzhang Wang[1,2], Jinwoo Ahn[2,3], Angela Gronenborn[2,3], and Tatyana Polenova[1,2]

[1]University of Delaware, Department of Chemistry and Biochemistry, Newark, DE; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 5th Ave., Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA

T36. Unbiased Genome Wide Screens for Host Cofactors Involved in Viral Restriction

Daniel W. Cyburt, Clifton L. Ricana, and Marc C. Johnson

Department of Molecular Microbiology and Immunology, University of Missouri-Columbia

Reverse Transcription

T37. Investigation of NMR Spectral Changes upon Homodimer Formation of HIV-1 Reverse Transcriptase

Ryan L. Slack[1], Naima G. Sharaf[1], Michael A. Parniak[2], Jinwoo Ahn[1], Angela M. Gronenborn[1], Rieko Ishima[1]
[1]Department of Structural Biology and [2]Department of Microbiology and Molecular Genetics, University of Pittsburgh, School of Medicine, PA-15260, USA

T38. Biochemical and Cellular Characterization of the SAMHD1 Ortholog, *Caenorhabditis Elegans* ZCK177.8

Lydia R. Studdard, Tatsuya Maehigashi and Baek Kim
Center for Drug Discovery, Emory School of Medicine, Pediatrics, Atlanta, GA

T39. Effect of Nucleic Acid Sequence on DNA Polymerization and NNRTI Inhibitory Mechanisms of HIV-1 Reverse Transcriptase

O Ukah[1], A Huber[2], E Michailidis[3], K Das[4], MA Parniak[5], K Singh[1], E Arnold[4], and S Sarafianos[1,6]
[1]University of Missouri, Molecular Microbiology and Immunology, Columbia, MO, United States; [2]University of Missouri, Veterinary Pathobiology, Columbia, MO, United States; [3]Rockefeller University, Virology and Infectious Disease, New York, NY, United States; [4]Rutgers University, Chemistry and Chemical Biology, Piscataway, NJ, United States; [5]University of Pittsburgh, Microbiology and Molecular Genetics, Pittsburgh, PA, United States; [6]University of Missouri, Biochemistry, Columbia, MO

T40. HIV-1 Capsid Facilitates Reverse Transcription by Retaining Reverse Transcriptase Within the Core

Janani Varadarajan and Christopher Aiken
Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232

T41. 3-Hydroxypyrimidine-2,4-diones as Novel HIV-1 RNase H Inhibitors

Karen A. Kirby[1,2], Jing Tang[3], Sanjeev K. V. Vernekar[3], Bulan Wu[3], Andrew D. Huber[1,4], Mary C. Casey[1,2], Juan Ji[1,2], Eva Nagy[5], Lena Miller[5], Qiongying Yang[1,2], Michael A. Parniak[5], Zhengqiang Wang[3], and Stefan G. Sarafianos[1,2,6]
[1]C.S. Bond Life Sciences Center, University of Missouri, Columbia, MO; [2]Dept. of Molecular Microbiology & Immunology, University of Missouri School of Medicine, Columbia, MO; [3]Center for Drug Design, University of Minnesota, Minneapolis, MN; [4]Dept. of Veterinary Pathobiology, University of Missouri School of Medicine, Columbia, MO; [5]Dept. of Microbiology & Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA; [6]Dept. of Biochemistry, University of Missouri, Columbia, MO

T42. Two Distinct Modes of Metal Ion Binding in the Nuclease Active Site of a Viral DNA-Packaging Terminase: Insight into the Two-Metal-Ion Catalytic Mechanism of RNaseH-like Nucleotidyltransferases

Haiyan Zhao[1], Zihan Lin[1], Anna Y. Lynn[1], Brittany Varnado[1], John A. Beutler[2], Ryan P. Murelli[3], Stuart F.J. Le Grice[4] and Liang Tang[1]

[1]Department of Molecular Biosciences, University of Kansas, 1200 Sunnyside Avenue, Lawrence, KS 66045; [2]Molecular Targets Laboratory, National Cancer Institute, Frederick, MD 21702; [3]Department of Chemistry, Brooklyn College, City University of New York, Brooklyn, NY 11210; [4]Basic Research Laboratory, National Cancer Institute, Frederick, MD 21702

T43. Characterization the C-Terminal Nuclease Domain of Herpes Simplex Virus Pul15 as a Target of Nucleotidyltransferase Inhibitors

Takashi Masaoka[1], Haiyan Zhao[2], Danielle R. Hirsch[3,4], Michael P. D'Erasmo[3,4], Christine Meck[3,4], Brittany Varnado[2], Marvin J. Meyers[5] Joel Baines[6], John A. Beutler[7], Ryan P. Murelli[3,4], Liang Tang[2] and Stuart F.J. Le Grice[1]

[1]Basic Research Laboratory, National Cancer Institute, Frederick, MD 21702, USA; [2]Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045, USA; [3]Department of Chemistry, Brooklyn College, City University of New York, Brooklyn, NY 11210, USA; [4]Department of Chemistry, The Graduate Center, City University of New York, New York, NY, 10016, USA; [5]Department of Chemistry, St. Louis University, St. Louis, MO 63103; [6]School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 USA; [7]Molecular Targets Laboratory, National Cancer Institute, Frederick, MD 21702, USA

Vif and APOBEC

TOC

T44. Molecular Characterization of a Unique Restriction Factor, APOBEC3H

Jennifer Bohn[1], Theodora Hatziioannou[2], and Janet Smith[1]

[1]Dept. of Biological Chemistry & Life Sciences Institute, University of Michigan, Ann Arbor, MI; [2]Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY

T45. NMR Structure of the APOBEC3B Catalytic Domain: Structural Basis for Substrate Binding and DNA Deaminase Activity

In-Ja L. Byeon[1], Chang-Hyeock Byeon[1], Tiyun Wu[2], Mithun Mitra[2], Dustin Singer[2], Judith G. Levin[2] and Angela M. Gronenborn[1]

[1]Department of Structural Biology and Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, United States; [2]Section on Viral Gene Regulation, Program in Genomics of Differentiation, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, United States

T46. The RNA Binding Specificity of Human APOBEC3 Proteins Mimics that of HIV-1 Nucleocapsid

Ashley York[1,2*], Sebla B Kutluay[3*], Manel Errando[4], and Paul D Bieniasz[1,2,5]

*[1]Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY; [2]The Rockefeller University, Laboratory of Retrovirology, New York, NY; [3]Washington University School of Medicine in St. Louis, Department of Molecular Microbiology, St. Louis, MO; [4]Department of Physics, Washington University in St. Louis, St. Louis, MO, [5]Howard Hughes Medical Institute, Aaron Diamond AIDS Research Center, New York, NY; *equal contributions*

T47. Identification of Recombinant Antibodies for Functional and Structural Analysis of HIV-Host Complexes

Natalia Sevillano[1], Hai Ta[1], Jennifer Binning[1], Florencia La Greca[1], Judd Hultquist[2], Nathalie Caretta[1], Amber Smith[3], Xuefeng Ren[4], Bei Yang[4], Bhargavi Jayaraman[1], Shumin Yang[1], Yifan Cheng[3], Nevan J. Krogan[2], John Gross[1] and Charles S. Craik[1]

[1]Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, USA; [2]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA; [3]Department of Biochemistry and Biophysics, University of California, San Francisco, CA, USA; [4]Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA

T48. VCBC-Specific Fabs Inhibit Ubiquitination and Degradation of A3 Proteins

Jennifer M. Binning[1], Amber Smith[4,5], Natalia Sevillano[1], Judd F. Hultquist[2,3], Nathalie Caretta Cartozo[1], Hai Ta[1], Nevan J. Krogan[2,3], Yifan Cheng[4,5], Charles S. Craik[1], John D. Gross[1]

[1]Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94158, USA; [2]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, California 94158, USA; [3]J. David Gladstone Institute, San Francisco, CA 94158; 4Howard Hughes Medical Institute, UCSF, San Francisco, CA 94158, USA; [4]Howard Hughes Medical Institute, UCSF, San Francisco, CA 94158, USA; [5]Department of Biochemistry and Biophysics, UCSF, San Francisco, CA 94158, USA

T49. Cleavable Cross-linkers, Multistage Mass Spectrometry, and the Structural Characterization of APOBEC3-Vif-CRL5 Complexes

Robyn M Kaake[1], Seung Joong Kim[3], Ignacia Echeverria[3], Linda Chelico[4], John D Gross[3], Andrej Sali[3], Lan Huang[5], Nevan J Krogan[1,2]

[1]The J David Gladstone Institutes, Institute for Virology and Immunology, San Francisco, CA; [2]University of California San Francisco, Dept. of Cellular and Molecular Pharmacology, San Francisco, CA; [3]University of California San Francisco, Dept. of Pharmaceutical Chemistry, San Francisco, CA; [4]University of Saskatchewan, College of Medicine, Saskatoon, Canada; [5]University of California Irvine, Dept. of Physiology & Biophysics, Irvine, CA

T50. Global Landscape of Ubiquitylation and Phosphorylation Changes in Response to HIV-1 Infection Identifies a Novel Substrate of Vif

Jeffrey Johnson[1,2], David Crosby[1], Judd Hultquist[1], Erik Verschuren[3], Lara Manganaro[4], Billy Newton[1], Tasha Johnson[1], Tricia Lundrigan[1], Viviana Simon[4], Alan Frankel[1], and Nevan Krogan[1,2*]

[1]University of California San Francisco; [2]Gladstone Institutes; [3]Genentech, Inc.; [4]Icahn School of Medicine at Mount Sinai; [*]Corresponding author

T51. Rerouting Resistance: Escaping Restriction Using Alternative Cellular Pathways

Aya Khwaja, Meytal Galilee, Ailie Marx, and Akram Alian

Faculty of Biology, Technion – Israel Institute of Technology, Haifa 320003, Israel

Nuclear Entry

TOC

T52. HIV-1 Interaction with CypA Regulates Use of FG-Nucleoporins for Nuclear Entry

Guangai Xue[1], Shih Lin Goh[2], Hyun Jae Yu[1], Anna T. Gres[3], KyeongEun Lee[1], Stefan G. Sarafianos[3], Jeremy Luban[2], and Vineet N. KewalRamani[1]
[1]Basic Research Laboratory, National Cancer Institute, Frederick, MD; [2]Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA; [3]Bond Life Sciences Center, MMI, Biochemistry, University of Missouri, Columbia, MO

T53. HIV-1 Nuclear Trafficking is Altered by Cytoplasmic CPSF6 Expression in a Capsid-Dependent Manner

Zhou Zhong[1], Callen Wallace[2], Christopher Kline[3], Simon C. Watkins[2], and Zandrea Ambrose[1,3]
[1]Molecular Virology and Microbiology Program, [2]Department of Cell Biology, and [3]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

Integrase

TOC

T54. HIV-1 Capsid Protein Modulates the Activity of Preintegration Complexes

Muthukumar Balasubramaniam[1], Amma Addai[1], Jing Zhou[2], Jui Pandhare[1], Christopher Aiken[2], and Chandravanu Dash[1]
[1]Center for AIDS Health Disparities Research, Meharry Medical College, Nashville, TN; [2]Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN

T55. Modeling Ligand Binding to an Allosteric Site in the Catalytic Core Domain of HIV-1 Integrase Using Absolute and Relative Free Energy Methods

Nanjie Deng,[1] Stefano Forli,[2] Joseph Bauman,[3] James Fuchs,[4] Mamuka Kvaratskhelia,[4] Alan Engelman,[5] Eddy Arnold,[3] Art Olson,[2] and Ronald Levy[1]
[1]Center for Biophysics and Computational Biology, Temple University, Philadelphia, PA; [2]The Scripps Research Institute, La Jolla, CA; [3]Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ; [4]College of Pharmacy, Ohio State University, Columbus, OH; [5]Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

T56. HIV-1 Integrase Strand Transfer Inhibitors that Reduce Susceptibility to Drug Resistant Mutant Integrases

Xue Zhi Zhao,[1] Hannah Peters,[1] Steven J. Smith,[2] Daniel P. Maskell,[4] Mathieu Metifiot,[3] Valerie E. Pye,[4] Katherine Fesen,[3] Christophe Marchand,[3] Yves Pommier,[3] Peter Cherepanov,[4,5] Stephen H. Hughes,[3] and Terrence R. Burke, Jr.[1]
[1]Chemical Biology Laboratory and [2]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD 21702; [3]Developmental Therapeutics Branch and Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; [4]Clare Hall Laboratories, The Francis Crick Institute, Blanche Lane, South Mimms, EN6 3LD, UK; [5]Imperial College London, St-Mary's Campus, Norfolk Place, London, W2 1PG, UK

T57. Selectivity for Strand-Transfer Over 3'-Processing and Susceptibility to Clinical Resistance of HIV-1 Integrase Inhibitors Are Both Driven by Key Enzyme-DNA Interactions in the Active Site

Mathieu Métifiot[1], Barry C. Johnson[2], Evgeny Kiselev[1], Laura Marler[1], Xue Zhi Zhao[3], Terrence R. Burke Jr[3], Christophe Marchand[1], Stephen H. Hughes[2] and Yves Pommier[1]
[1]Developmental Therapeutics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; [2]HIV Drug Resistance Program, Frederick National Laboratory for Cancer Research, Center for Cancer Research, National Cancer Institute-Frederick, National Institutes of Health, Frederick, MD 21702; [3]Chemical Biology Laboratory, Frederick National Laboratory for Cancer Research, Center for Cancer Research, National Cancer Institute-Frederick, National Institutes of Health, Frederick, MD 21702

T58. Using Fragment-Based Analysis of Virtual Screenings to Characterize Binding Sites

Richard K. Belew[1], Stefano Forli[2], David Goodsell[2], T. J. O'Donnell[3], and Arthur Olson[2]
[1]Univ. California – San Diego (rbelew@ucsd.edu); [2]The Scripps Research Institute; [3]gNova

TOC

Vpr

T59. Structure-Selective Endonuclease MUS81/EME1 Downregulation and G2 Cell Cycle Arrest are Independent Functions of Vpr

Xiaohong Zhou, Maria DeLucia, and Jinwoo Ahn
Department of Structural Biology and Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA

T60. To Repair or Not Repair: The X-ray Structure of the DDB1-DCAF1-Vpr-UNG2 Complex

Ying Wu[1], Xiaohong Zhou[1], Christopher O. Barnes[2], Maria DeLucia[1], Aina E. Cohen[3], Angela M. Gronenborn[1], Jinwoo Ahn[1], and Guillermo Calero[1]*
*[1]Department of Structural Biology and Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA; [2]Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA; [3]Stanford Synchrotron Radiation Lightsource, Menlo Park, CA 94025, USA; *Correspondence: jia12@pitt.edu (J.A.), guc9@pitt.edu (G.C.)*

TOC

Provirus and Transcription

T61. Human T-Cell Leukemia Virus Type 1 Proviral Load and Genome Structure in Chronically Infected T-Cell Lines

Morgan E Meissner[1,2], LeAnn Oseth[5], Jessica L Martin[1,3], Luiza Mendonca[1], Wei Zhang[1,4], Louis M Mansky[1,2,3]
[1]University of Minnesota, Institute for Molecular Virology, Minneapolis, MN, [2]University of Minnesota, Molecular, Cellular, Developmental Biology, and Genetics Graduate Program, Minneapolis, MN, [3]University of Minnesota, Pharmacology Graduate Program, Minneapolis, MN, [4] University of Minnesota, Characterization Facility, Minneapolis, MN, [5]University of Minnesota, Cytogenomics Core, Minneapolis, MN

T62. Structural Basis of an Evolved Cre Recombinase that Excises HIV DNA

Gretchen Meinke[1], Janet Karpinski[2], Frank Buchholz[2] and Andrew Bohm[1]
[1]Department of Developmental, Molecular and Chemical Biology, Tufts University School of Medicine, Boston, MA, 02111, USA; [2]Medical Systems Biology, UCC, Medical Faculty Carl Gustav Carus, TU Dresden, Germany

T63. DNA-PK Inhibition Potently Represses HIV Transcription and Replication

Geetaram Sahu[1], Kalamo Farley[1], Gary Simon[1] and Mudit Tyagi[1, 2]
[1]Division of Infectious Diseases, Department of Medicine, George Washington University, Washington, DC 20037; [2]Department of Microbiology, Immunology and Tropical Medicine, George Washington University, Washington, DC 20037

T64. Identification of Smac Mimetics as a Novel Class of HIV-1 Latency Reversing Agents

Lars Pache[1], Miriam S. Dutra[1], Adam M. Spivak[2], John M. Marlett[3], Jeffrey P. Murry[3§], Young Hwang[4], Ana M. Maestre[5], Lara Manganaro[5], Mitchell Vamos[1], Peter Teriete[1], Laura J. Martins[2], Renate König[1,6], Viviana Simon[5], Alberto Bosque[2], Ana Fernandez-Sesma[5], Nicholas D. P. Cosford[1], Frederic D. Bushman[4], John A. T. Young[3#], Vicente Planelles[2] and Sumit K. Chanda[1]
[1]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037; [2]University of Utah School of Medicine, Salt Lake City, UT 84112; [3]The Salk Institute for Biological Studies, La Jolla, CA 92037; [4]Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104; [5]Icahn School of Medicine at Mount Sinai, New York, NY 10029; [6]Paul-Ehrlich-Institut, 63225 Langen, Germany; [§]Present address: Gilead Sciences, Foster City, CA 94404; [#] Present address: F. Hoffmann-La Roche Ltd, 4070 Basel, Switzerland

T65. Visualization of Transcriptional Activation from HIV Integration Sites in Latently Infected Cells

Obiaara Ukah[1,2], Maritza Puray-Chavez[1,2], Juan Ji[1], Stefan G Sarafianos[1,2,3,4]
[1]University of Missouri, C.S. Bond Life Sciences Center, Columbia, MO, [2]University of Missouri, Molecular Microbiology & Immunology, Columbia, MO, [3]University of Missouri, Veterinary Pathobiology, Columbia, MO, [4]University of Missouri, Biochemistry, Columbia, MO

T66. Heterogenic Transcription Start Sites of HIV-1 and Their Influence on RNA Fates

Siarhei Kharytonchyk[1], Philip Smaldino[1], Sarah Monti[2], Michael F. Summers[2], and Alice Telesnitsky[1]
[1]Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor MI 48109-0620; [2]Howard Hughes Medical Institute and Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

T67. Insights into HIV-1 Proviral Transcription from an Integrative Structure of the Tat:AFF4:P-Tefb:TAR Complex

Ursula Schulze-Gahmen[1], Ignacia Echeverria[2], Goran Stjepanovic[1], Yun Bai[1], Huasong Lu[1], Dina Schneidman-Duhovny[2], Jennifer A. Doudna[1,3,4,5], Qiang Zhou[1], Andrej Sali[2], and James H. Hurley[1,5]

[1]Department of Molecular and Cell Biology and California Institute of Quantitative Biosciences, University of California, Berkeley, Berkeley, United States; [2]Department of Bioengineering and Therapeutic Sciences, Department of Pharmaceutical Chemistry, and California Institute of Quantitative Biosciences, University of California San Francisco, San Francisco, United States; [3]Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, United States; [4]Department of Chemistry, University of California, Berkeley, Berkeley, United States; [5]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, United States

T68. Probing Structural Dynamics and Kinetics of HIV-1 RNA Recognition to Guide RNA Drug Design

Nicole Orlovsky, Isaac Kimsey, Terrence Oas, and Hashim M. Al-Hashimi

Department of Biochemistry, Duke University, Durham, NC

T69. Structure Based Methods to Target HIV-1 Transactivation Response Element RNA

Laura R. Ganser[1], Janghyun Lee[4], Bharathwaj Sathyamoorthy[5], Hal P. Bogerd[2], Yi Xue[1], Dawn K. Merriman[3], Aman Kansal[1], Paul Bieniasz[6], Bryan R. Cullen[2], Hashim M. Al-Hashimi[1]

[1]Department of Biochemistry, [2]Department of Molecular Genetics, and [3]Department of Chemistry, Duke University, Durham, NC 27710; [4]Department of Chemistry, Korea Advanced Institute of Science and Technology, Deejeon, 34141 South Korea; [5]Department of Chemistry, Indian Institute of Science Education and Research Bhopal, Bhopal 462-066, India; [6]Aaron Diamond AIDS ResearchCenter, Laboratory of Retrovirology and Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10016

T70. Virtual Screening Aided Design, Synthesis and SAR Study on Amiloride Derivatives as Probes for HIV-1 RNA

Umuhire Juru, A.[1], Patwardhan, N.N.[1], Eubanks, C.S.[1], Kapral G.J.[1], Lee, J. [2], Ganser, L.[2], Sathyamoorthy B.[2], Al-Hashimi, H. [2], and Hargrove, A.E.[1]

[1]Department of Chemistry, Duke University, Durham, NC; [2]Department of Biochemistry, Duke University School of Medicine, Durham, NC

Friday Posters

RNA Splicing

F1. A "U2AF Homology Motif" of Tat-SF1, a Host Cofactor for HIV-1 RNA Splicing, Recognizes the Human SF3b1 Spliceosome Subunit and the HIV-1 Rev Protein

Steven Horner, Sarah Loerch, and Clara L. Kielkopf
Center for RNA Biology, University of Rochester School of Medicine & Dentistry, Rochester, NY 14642

F2. Combined NMR and SAXS Studies of HIV and SIV Splicing Elements

Christopher E. Morgan, Niyati Jain, and Blanton S. Tolbert
Department of Chemistry, Case Western Reserve University, Cleveland, OH

F3. Splicing in a Panel of HIV-1 Transmitted/Founder Virus

Ann Emery[1] and Ronald Swanstrom[2]
[1]Genetics and Molecular Biology Curriculum, [2]Department of Biochemistry and Biophysics, University of North Carolina Chapel Hill

F4. RNA Specificity of HIV Splicing Factor Revealed by Global Analysis of its Binding Landscape

Niyati Jain, Hsuan-Chun Lin, Christopher E. Morgan, Michael E. Harris, and Blanton S. Tolbert
Case Western Reserve University

F5. Global Synonymous Mutagenesis Identifies Novel *Cis*-Acting RNA Sequences that Regulate HIV-1 Splicing and Replication

Matthew Takata[1,2], Steven Soll[1,2,3], Ann Emery[4,5], Daniel Blanco-Melo[1,2], Ronald Swanstrom[4,5] and Paul D. Bieniasz[1,2,3]
[1]Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY; [2]The Rockefeller University, Laboratory of Retrovirology, New York, NY; [3]Howard Hughes Medical Institute, Aaron Diamond AIDS Research Center, New York, NY; [4]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [5]Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

RRE and Rev

F6. Structure-Function Studies on the HIV-1 Rev Response Element

Ina O'Carroll[1,2], Yashna Thappeta[1], Lixin Fan[3], Edric Ramirez-Valdez[1], Sean Smith[1], Yun-Xing Wang[3], Alan Rein[1]
[1]HIV Dynamics and Replication Program, National Cancer Institute, Frederick, MD; [2] Chemistry Department, United States Naval Academy, Annapolis, MD; [3]National Cancer Institute, Structural Biophysics Laboratory, Frederick, MD

F7. Role of DEAD-box Protein DDX1 in Assembly of Rev-RRE Nuclear Export Complexes

Rajan Lamichhane, John Hammond, Raymond Pauszek, Ingemar Pedron, Edwin van der Schans, James R. Williamson and David P. Millar

Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla CA 92037

F8. Cryo-Electron Microscopy of HIV-1 RNAs

Zhaoming Su[1], Kaiming Zhang[1], Muyuan Chen[1], Sarah Keane[2], Jan Marchant[2], Steve Ludtke[1], Michael Schmid[1], Michael F. Summers[2], and Wah Chiu[1]

[1]National Center for Macromolecular Imaging, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030; [2]Howard Hughes Medical Institute and Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Cricle, Baltimore, Maryland 21250

RNA Structure

TOC

F9. A Combined Chemical and Phylogenetic Approach for HIV-1 and SIV RNA Secondary Structure Prediction Within and Among Infected Hosts

Brittany D. Rife[1,2], Carla N. Mavian[1,2], Susanna L. Lamers[3], David J. Nolan[1,2], Sergei Kosakovsky Pond[4], and Marco Salemi[1,2]

[1]Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL, USA; [2]Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA; [3]Bioinfoexperts, LLC, Thibodaux, LA, USA; [4]Department of Biology, Temple University, Philadelphia, PA, USA.

F10. Impact of the HLA B*57 Allele on Intra-Host HIV-1 Capsid-Coding RNA Secondary Structure Diversity

Brittany D. Rife[1,2], Carla N. Mavian[1,2], Susanna L. Lamers[3], David J. Nolan[1,2], Frederick M. Hecht[5], Annika C. Karlsson[4], and Marco Salemi[1,2]

[1]Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL, USA; [2]Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA; [3]Bioinfoexperts, LLC, Thibodaux, LA, USA; [4]Karolinska Institutet, Stockholm, Sweden; [5]UCSF Positive Health Program, San Francisco General Hospital, University of California, San Francisco, California, USA

F11. Conserved Global Structure and Function of Genomic RNA 5´-UTR Across Prototypic HIV-1 Subtypes

Roopa Comandur, Erik D. Olson, William A. Cantara, Joshua E. Hatterschide, Christopher P. Jones, and Karin Musier-Forsyth

Department of Chemistry and Biochemistry, Center for Retrovirus Research and Center for RNA Biology, The Ohio State University, Columbus, OH 43210

F12. HIV-1 Translation is Positively Regulated by Higher Order Conformation of 5'RNA

Ioana Boeras[1], Zhenwei Song[2], Xiao Heng[2], Sarah Monti[3], Aaron Rendahl[1], and Kathleen Boris-Lawrie[1]
[1]University of Minnesota, Saint Paul, MN; [2]University of Missouri, Columbia, MO; [3]University of Maryland, Baltimore County, Baltimore, MD

F13. Identification of the Extended Dimer Interface of the HIV-1 5′ Leader

Sarah C. Keane,[1] Verna Van,[1] Heather M. Frank,[1] Carly Sciandra,[1] Sayo McCowin,[1] Justin Santos,[1] Xiao Heng,[2] and Michael F. Summers[1]
[1]Howard Hughes Medical Institute (HHMI) and Department of Chemistry and Biochemistry, University of Maryland Baltimore County (UMBC). 1000 Hilltop Circle, Baltimore, MD 21250, USA; [2]Department of Biochemistry, University of Missouri, Columbia, MO, 65211, USA

F14. Identifying Conserved RNA Binding Motifs for PSF/SFPQ, a Critical Host Factor for HIV-1 Replication

Gatikrushna Singh[1], Brittany Rife[2], Marco Salemi[2], Leslie Parent[3] and Kathleen Boris-Lawrie[1]
[1]University of Minnesota, Saint Paul, MN; [2]University of Florida, Gainesville, FL; [3]Penn State College of Medicine, Hershey, PA

F15. Renal Risk Variants of APOL1 RNA Contribute to Podocyte Injury by Activating Protein Kinase R

Koji Okamoto[1], Jason W. Rausch[2], Joon-Yong Chung[3], Avi Z. Rosenberg[4], Stephen M. Hewitt[3], Eisei Noiri[5], Stuart F.J. Le Grice[2], Maarten Hoek[6], Cheryl A. Winkler[7] and Jeffrey B. Kopp[1]
[1]Kidney Disease Section, NIDDK, NIH, [2]Reverse Transcriptase Biochemistry Section, Basic Research Program, FNLCR; [3]Experimental Pathology Lab, Laboratory of Pathology, Center for Cancer Research, NCI, NIH; [4]Department of Pathology, Johns Hopkins Medical Institutions; [5]Department of Nephrology, Endocrinology, Hemodialysis & Apheresis, University Hospital, The University of Tokyo; [6]Merck Research Laboratories, Merck and Co.; [7]Basic Research Laboratory, Center for Cancer Research, NCI, Leidos

F16. Structural Characterization of Large RNAs from HIV-1 Using NMR

Jan Marchant[1], Roald Teuben[1], Lindsay Glang[1], Geraldine Ezeka[1], Michael Lopresti[1], Kaiming Zhang[2], Zhaoming Su[2], Wah Chiu[2] and Michael Summers[1]
[1]Howard Hughes Medical Institute, University of Maryland Baltimore County; [2]Baylor College of Medicine

F17. Development of Site-Specifically Labeled Nucleotides to Address Problems in NMR Spectroscopy

Andrew P. Longhini, Regan M. Leblanc, and Theodore K. Dayie
University of Maryland, College Park

F18. Variation in KSHV-Encoded microRNA Sequence Affect the Levels of Mature microRNAs in Kaposi Sarcoma Lesions

Vickie Marshall[1], Nazzarena Labo[1], Joanna Sztuba-Solinska[2], Elena M Cornejo-Castro[1], Karen Aleman[3], Kathleen M Wyvill[3], Lynne McNamara[4], Stuart FJ Le Grice[2], Robert Yarchoan[3], Thomas S Uldrick[3], Mark N Polizzotto[3], Patrick MacPhail[4] and Denise Whitby[1]
[1]AIDS and Cancer Virus Program, Leidos Biomedical, Frederick National Laboratory for Cancer Research, Frederick MD 21702; [2]Basic Research Program, National Cancer Institute, Frederick, MD 21702; [3]HIV and AIDS Malignancy Branch, National Institutes of Health, Bethesda, MD; [4]Clinical HIV Research Unit, Department of Internal Medicine, University of the Witwatersrand, Johannesburg, South Africa

TOC

RNA Packaging

F19. 5′ Start Site Heterogeneity of the HIV-1 RNA and its Effect on Structure and Function

Joshua Brown, Seungho Choi, Michael Lopresti, Hannah Carter, Aishwarya Iyer, Jana Hiji, Nicolas Bolden, Lindsay Glang, and Michael Summers
University of Maryland Baltimore County

F20. DHX9/RHA Binding to the PBS-Segment of the Genomic RNA during HIV-1 Assembly Bolsters Virion Infectivity

Ioana Boeras[1], Zhenwei Song[2], Andrew Moran[2], Jarryd Franklin[2], William Clay Brown[3], Marc Johnson[4], Kathleen Boris-Lawrie[1] and Xiao Heng[2]
[1]Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN 55108, USA; [2]Department of Biochemistry, University of Missouri, Columbia, MO 65211, USA; [3]Center for Structural Biology, Life Sciences Institute, University of Michigan, Ann Arbor, MI 48109, USA; [4]Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO 65211, USA

F21. RNA Structure Provides Insights into Mechanism of Selective Genome Packaging by Retroviral Gag

Erik D Olson[1], Tiffiny Rye-McCurdy[1], Brian R Thompson[1], Ioulia Rouzina[1], Leslie Parent[2], and Karin Musier-Forsyth[1]
[1]Department of Chemistry and Biochemistry, Center for RNA Biology, and Center for Retroviral Research, The Ohio State University, Columbus, OH; [2]Penn State College of Medicine, Departments of Medicine and Microbiology and Immunology, Hershey, PA

F22. Probing HTLV-1 Matrix-Viral Genomic RNA Interactions

William A. Cantara, Weixin Wu, and Karin Musier-Forsyth
Department of Chemistry and Biochemistry, Center for RNA Biology, and Center for Retroviral Research, The Ohio State University, Columbus, OH 43210

F23. Cellular MicroRNAs are Packaged into HIV-1 Virions

Hal P. Bogerd[1], Edward M. Kennedy[1], Adam W. Whisnant[2] and Bryan R. Cullen[1]
[1]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710, USA; [2] Institute of Virology, University of Würzburg, Würzburg, Germany

F24. In Vitro Selective Binding between the HIV-1 Packaging Signal and Gag is Driven by a Delicate Balance between Specific and Non-Specific Interactions

Mauricio Comas-Garcia[1], Siddhartha A.K. Datta[1], Rajat Varma[2] and Alan Rein[1]
[1]HIV DRP, National Cancer Institute, Frederick, MD 21702, USA; [2]Laboratory of Systems Biology, National Institute of Allergic and Infectious Diseases, Bethesda, MD, 20892

F25. HIV-1 Gag Co-Localization with Unspliced vRNA in the Nucleus Occurs During or Shortly After Transcription

Kevin M. Tuffy[1], Rebecca J. Kaddis Maldonado[1], Breanna Rice[1], Alan Cochrane[3], and Leslie J. Parent[1,2]
[1]Departments of Medicine and [2]Microbiology & Immunology, Penn State College of Medicine, Hershey, PA; [3]Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada

F26. Readily Accessible Multiplane Microscopy: 3D Tracking the HIV-1 Genome in Living Cells

Michelle S. Itano, Marina Bleck, Daniel S. Johnson, and Sanford M. Simon
Laboratory of Cellular Biophysics, The Rockefeller University, New York, NY

F27. Biochemical Reconstitution of Selective HIV-1 Genome Packaging

Lars-Anders Carlson[1], Yun Bai[1,3], Sarah C. Keane[2], Jennifer A. Doudna[1], and James H. Hurley[1]
[1]Department of Molecular and Cell Biology, University of California, Berkeley; [2]University of Maryland, Baltimore County; [3]Current address: School of Life Science and Technology, ShanghaiTech University, Shanghai, 201210, China

F28. Screening Potential Small Molecule Inhibitors Against the Core Encapsidation Signal of HIV Using Nuclear Magnetic Resonance

Julie Nyman, Jessica Zaki, and Michael Summers
UMBC, Department of Chemistry and Biochemistry

TOC

Gag Trafficking and Assembly

F29. Characterizing the Host Cell Factors Involved in HIV-1 Gag Trafficking to Sites of Virus Assembly

Rachel Van Duyne, Philip R. Tedbury, and Eric O. Freed
Virus-Cell Interaction Section, HIV Dynamics and Replication Program, NCI-Frederick, MD

F30. Retroviral Gag Puncta Biogenesis and Quantitative Measurements of Gag Stoichiometry

Isaac Angert, John Eichorst, Jessica L Martin, Wei Zhang, Louis M Mansky, and Joachim D Mueller
Institute for Molecular Virology, University of Minnesota, Minneapolis, MN 55455

F31. HTLV-1 and HIV-1 CA-CA Interactions Involved in Virus Particle Assembly

Jessica L Martin, Rachel Marusinec, Louis M Mansky
University of Minnesota, Institute for Molecular Virology, Minneapolis, MN

F32. Design and Characterization of Enveloped Protein Nanoparticles

Jörg Votteler[1], Cassie Ogohara[2], Sue Yi[2], David Belnap[1], David Baker[2], Neil P. King[2], and Wesley I. Sundquist[1]

[1]CHEETAH Center and Department of Biochemistry, University of Utah, Salt Lake City, Utah 84113; [2]Institute for Protein Design, University of Washington, Seattle, Washington 98195

F33. Virus-like Particles of Immature HIV-1 Assembled on Bacteriophage-derived Templates

Pooja Saxena[1], Li He[2†], Andrey Malyutin[1‡], Siddhartha A. K. Datta[3], Alan Rein[3], Kevin M. Bond[1], Martin F. Jarrold[1], Alessandro Spilotros[4], Dmitri Svergun[4], Trevor Douglas[1] and Bogdan Dragnea[1]

[1]Department of Chemistry, Indiana University, Bloomington, IN 47405, USA; [2]Department of Molecular and Cellular Biochemistry, Indiana University, Bloomington, IN 47405, USA; [3]National Cancer Institute, P.O. Box B, Building 535, Frederick, MD 21702-1201, USA; [4]European Molecular Biology Laboratory-DESY, Notkestrasse 85, Geb. 25a, 22603 Hamburg, Germany; Present addresses: [†]Department of Genetics, Yale School of Medicine, 333 Cedar Street, New Haven, CT 06510; [‡]Columbia University Medical Center, Department of Biochemistry and Molecular Biophysics, 650 W 168th St, Black Building 2-221, New York, NY 10032

TOC

Gag-Membrane Interaction

F34. Matrix Mutations Responsible for Retargeting Gag to MVBs also Decrease Matrix's Affinity for $tRNA^{Lys3}$

Christy R. Gaines, Amalia Rivera-Oven, Emre Tkacik, Ally Yang, Alecia Achimovich, Tawa Alabi, and Michael Summers

Howard Hughes Medical Institute at UMBC

F35. HIV-1 Matrix-31 Membrane Binding Peptide Interacts Differently with Membranes Containing PS vs. $PI(4,5)P_2$

Lauren O'Neil[1], Kathryn Andenoro[1], Isabella Pagano[1], Laura Carroll[1], Leah Langer[1], Zachary Dell[1], Davina Perera[2], Bradley W. Treece[1], Frank Heinrich[1,3], Mathias Lösche[1,3,4], John F. Nagle[1] and Stephanie Tristram-Nagle[1]

[1]Biological Physics Group, Physics Department, Carnegie Mellon University, Pittsburgh, PA 15213; [2]Biomedical Engineering, Douglass College, Rutgers University, New Brunswick, NJ 08901; [3]National Institute of Standards and Technology Center for Neutron Research, Gaithersburg, MD 20899; [4]Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213

F36. Cholesterol Enhancement of Retroviral Gag Protein-Membrane Interaction: Mechanistic Insights

Milka Doktorova[1], Fred Heberle[2,3], Richard Kingston[4], John Katsaras[3], Gerald Feigenson[5], Harel Weinstein[6], Volker Vogt[5], and Robert Dick[5]

[1]Weill Cornell Medical College, Tri-Institutional Program in Computational Biology and Medicine; [2]Oak Ridge National Laboratory, Biology and Soft Matter Division; [3]University of Tennessee, Bredesen Center for Interdisciplinary Research and Graduate Education; [4]School of Biological Sciences, University of Auckland; [5]Cornell University, Department of Molecular Biology and Genetics; [6]Weill Cornell Medical College, Department of Physiology and Biophysics

F37. Molecular Determinants of Retroviral Gag Membrane Assembly

Frank Heinrich,[1,2] Marilia Barros,[1] Rebecca Eells,[1] Ioannis Karageorgos,[3,4] Hirsh Nanda,[1,2] Robert A. Dick,[5] Volker M. Vogt,[5] Sid A. K. Datta,[6] Alan Rein,[6] and Mathias Lösche[1,2]
[1]Dept. of Physics and Dept. of Biomedical Engineering, Carnegie Mellon University; [2]The NIST Center for Neutron Research; [3]Biomolecular Measurement Division, NIST; [4]Institute for Bioscience and Biotechnology Research (IBBR); [5]Dept. of Molecular Biology and Genetics, Cornell University; [6]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, NIH

F38. Membrane Charge and Order Influence Membrane Binding of the Retroviral Structural Protein Gag

Yi Wen, Robert A. Dick, Gerald W. Feigenson and Volker M. Vogt
Department of Molecular Biology and Genetics, Cornell University, Ithaca NY 14853

TOC

Protease and Inhibition

F39. Co-Crystallization of Nucleotides with HIV Protease Reveals a Potential Mechanism for Rate Enhancement in the Presence of RNA

Tiefenbrunn, T[1]., Happer, M.[2], Lin, Y.-C.[2], Elder, J. H.[2], Stout, C. D.[1,*]
*Departments of [1]ICSB, [2]IMS, TSRI, 10550 N. Torrey Pines Rd., La Jolla, CA 92037; *Deceased April 2016*

F40. Analyzing the Hydration Structure of HIV-1 Protease using Molecular Dynamics Simulations

Florian Leidner,[1] Janet Paulsen,[1] Nese KurtYilmaz,[1] Yves Muller,[2] and Celia Schiffer[1]
[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, USA; [2]Division of Biotechnology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

F41. Design, Stereoselective Synthesis and Evaluation of HIV-1 Protease Inhibitors Incorporating Novel P2′ Groups

Linah N. Rusere,[1] Akbar Ali,[1] Sook-Kyung Lee,[2] Ronald Swanstrom,[2] and Celia A. Schiffer[1]
[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605, United States; [2]Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, United States

F42. Long-Range Structural Perturbation upon Active-Site Inhibitor Interaction on HIV-1 Protease

Shahid N Khan, John D Persons, Michel T Guerrero, and Rieko Ishima
Department of Structural Biology, University of Pittsburgh School of Medicine

F43. Exploring Surface Sites on HIV Protease as Targets for Inhibitors: From Computation to Biological Activity

Tiefenbrunn, T.[1], Forli, S.[1], Happer, M.[2], Gonzalez, A[3]., Baksh, M. M.[4], Chang, M. W.[5], Tsai, Y.-S.[3], Lin, Y.-C.[2], Perryman, A. L.[1], Rhee, J.-K.[4], De Vera, I.[6], Kojetin, D.[6], Torbett, B.E.[5], Olson, A. J.[1], Soltis, M.[3], Elder, J. H.[2], Finn, M. G.[4], and Stout, C. D.[1,*]

*Departments of [1]ICSB, [2]IMS, [5]MEM, TSRI, 10550 N. Torrey Pines Rd., La Jolla, CA 92037; [3]SSRL, SLAC, 2575 Sand Hill Road MS 99, Menlo Park, CA 95124; [4]School of Chemistry & Biochemistry, Georgia Tech, 901 Atlantic Drive, Atlanta, GA 30332; [6]Department of Molecular Therapeutics, TSRI Florida, 130 Scripps Way, Jupiter, FL 33458; *Deceased April 2016*

F44. Interdependence of Inhibitor Recognition in HIV-1 Protease Sub-Sites

Janet Paulsen, Florian Leidner, Akbar Ali, and Celia Schiffer

Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, USA

F45. Improving Inhibitor Design to Counter Drug Resistance: Lessons from HIV-1 and HCV Protease Inhibitors

Nese Kurt Yilmaz[1], Ronald Swanstrom[2] and Celia A. Schiffer[1]

[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605, USA; [2]Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA

F46. Mapping The Fitness Landscapes of Drug-Resistance in HIV Protease

Jeffrey I Boucher[1], Troy W Whitfield[1], Sook-Kyung Lee[3,4], Ronald Swanstrom[2,3,4], Celia A Schiffer[1], and Daniel NA Bolon[1]

[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester MA 01605, USA; [2]Department of Microbiology and Immunology, [3]Department of Biochemistry and Biophysics, and the [4]UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

F47. The HIV-1 Late Domain-2 S40A Polymorphism in Antiretroviral Exposed Individuals Influences Protease Inhibitor Susceptibility

Susan M Watanabe[1], Viviana Simon[2], Brittney R Kemp[3], Satoshi Machihara[3], Kimdar Sherefa Kemal[4], Binshan Shi[5], Brian Foley[6], Barbara Weiser[7,8], Harold Burger[7,8], Kathryn Anastos[4], Chaoping Chen[3], and Carol A Carter[1]

[1]Stony Brook University, Dept. of Molecular Genetics & Microbiology, Stony Brook, NY; [2]Icahn School of Medicine at Mount Sinai, Dept. of Microbiology, Global Health and Emerging Pathogens Institute, New York, NY; [3]Colorado State University, Dept. of Biochemistry and Molecular Biology, Denver, CO; [4]Albert Einstein College of Medicine, Dept. of Medicine, New York, NY; [5]Albany College of Pharmacy and Health Sciences, Dept. of Health Sciences, Albany, NY; [6]Los Alamos National Laboratory, Theoretical Biology and Biophysics Dept., Los Alamos, NM; [7]University of California Davis, Dept. of Medicine, Sacramento, CA; [8]Sacramento VA Medical Center, Dept. of Medicine, Sacramento, CA

F48. The Contribution of Mutations Outside of the Protease Appeared During DRV Selection in Conferring Resistance to Highly Potent Protease Inhibitors

Sook-Kyung Lee[1], Ean Spielvogel[1], Shuntai Zhou[1], Jesse Crayle[1], Celia Schiffer[2], and Ronald Swanstrom[1]

[1]Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina, Chapel Hill, NC; [2]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts, Worcester, MA

F49. Effects of Natural Polymorphisms of non-B HIV-1 Protease on Protein Conformations

Trang Tran[1], Zhanglong Liu[1], Ben M. Dunn[2], and Gail E. Fanucci[1]

[1]Department of Chemistry, University of Florida, Gainesville, FL 32611; [2]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610

F50. Inference of Epistatic Effects and the Development of Drug Resistance in HIV-1 Protease

William F. Flynn[1,2], Allan Haldane[2], and Ronald M. Levy[2]

[1]Department of Physics and Astronomy, Rutgers University, Piscataway, NJ; [2]Center for Biophysics and Computational Biology, Department of Chemistry, Temple University, Philadelphia, PA

F51. Altering the Conformational Landscape as a Mechanism for Evolution in HIV-1 Protease

Zhanglong Liu, Lingna Hu, Linh Pham, Trang T. Tran, Xi Huang, Ben M. Dunn, and Gail E. Fanucci

Department of Chemistry, University of Florida, Gainesville, FL 32611

F52. Selection to Confirm Novel Resistance Pathways to Potent New UMASS HIV-1 Protease Inhibitor

Ean Spielvogel[1], Sook-Kyung Lee[1], Shuntai Zhou[1], J Paulsen[2], Celia Schiffer[2], and Ronald Swanstrom[1]

[1]Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina, Chapel Hill, NC; [2]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts, Worcester, MA

F53. Mechanistic Characterization of Inhibitor Resistance in HIV-1 Protease via Machine Learning and Atomistic Simulation

Debra A. Ragland[1], Troy W. Whitfield[2,3], Nese Kurt-Yilmaz[1], Konstantin Zeldovich[1,3] and Celia A. Schiffer[1]

[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester MA 01655; [2]Department of Medicine, University of Massachusetts Medical School, Worcester MA 01655; [3]Program in Bioinformatics and Integrative Biology, University of Massachusetts Medical School, Worcester MA 01655

Maturation

TOC

F54. The Race Against Protease Activation Defines the Role of ESCRTs in HIV Budding

Mourad Bendjennat[1] and Saveez Saffarian[1,2,3,#]
[1]Department of Physics and Astronomy, University of Utah, Salt Lake City, Utah, USA; [2]Center for Cell and Genome Science, University of Utah, Salt Lake City, Utah, USA; [3]Department of Biology, University of Utah, Salt Lake City, Utah, USA; [#]Saffarian@physics.utah.edu

T55. Resistance Pathways for Potent and Broadly Active HIV-1 Maturation Inhibitors; Insights into Gag Structure During Assembly and Maturation

Emiko Urano[1], Sherimay D. Ablan[1], Justin Kaplan[1], Nishani Kuruppu[1], Juan Fontana[2], Alasdair C. Steven[2], Mingzhang Wang[3], Caitlin Quinn[3], Tatyana Polenova[3], David E. Martin[4], T. J. Nitz[4], Carl T. Wild[4], and Eric O. Freed[1]
[1]HIV Dynamics and Replication Program, NCI, Frederick, MD; [2]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE; [3]NIAMS, NIH, Bethesda, MD; [4]DFH Pharma, Inc., Gaithersburg, MD

F56. Dynamic Regulation of HIV-1 Capsid Maturation by Integrated Magic Angle Spinning NMR and Molecular Dynamics Simulations

Mingzhang Wang[1,2], Caitlin M. Quinn[1,2], Juan R. Perilla[4], Huilan Zhang[1,2], Guangjin Hou[1], Rupal Gupta[1,2], Sherimay Ablan[5], Emiko Urano[5], Jinwoo Ahn[2,3], In-Ja Byeon[2,3], Christopher Aiken[2,6], Klaus Schulten[4], Angela M. Gronenborn[2,3], Eric O. Freed[5], and Tatyana Polenova [1,2]
[1]University of Delaware, Department of Chemistry and Biochemistry, Newark, DE; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 5th Ave., Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA; [4]University of Illinois, Theoretical and Computational Biophysics Group, Urbana, IL; [5]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Frederick, MD; [6]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN

F57. Identification of a Novel Element in HIV-1 CA Critical for Assembly and Maturation

Mariia Novikova[1], Muthukumar Balasubramaniam[1], Sagar Kudchodkar[1], Ferri Soheilian[2], Anna T Gres[3], Karen A Kirby[3], Juan Fontana[4], Alasdair C. Steven[4], Stefan G Sarafianos[3], and Eric O Freed[1]
[1]Virus-Cell Interaction Section, HIV Dynamics and Replication Program, NCI-Frederick, Frederick, MD; [2]Electron Microscope Laboratory, Cancer Research Technology Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD; [3]University of Missouri, C.S. Bond Life Sciences Center, Columbia, MO; [4]Laboratory of Structural Biology Research, National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, MD

F58. Crystal Structure of an HIV Assembly and Maturation Switch

Jonathan Wagner, Kaneil Zadrozny, Jakub Chrustowicz, Michael Purdy, Mark Yeager, Barbie Ganser-Pornillos, and Owen Pornillos
Department of Molecular Physiology and Biological Physics, University of Virginia School of Medicine

F59. Structural Analysis of CA-SP1-NC Assemblies by Magic Angle Spinning NMR

Ryan W. Russell[1,2], Christopher L. Suiter[1,2], Guangjin Hou[1,2], Caitlin M. Quinn[1,2], Angela M. Gronenborn[2,3], Mingzhang Wang[1,2], Manman Lu[1,2], Jinwoo Ahn[2,3], Sherimay D. Ablan[4], Emiko Urano[4], Eric O. Freed[4], and Tatyana Polenova[1,2]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 5th Ave., Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA; [4]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Frederick, MD

F60. Dynamic Characterization of The Spacer Peptide 1 (SP1) in Immature HIV-1 Capsid Protein Assemblies

Juan R Perilla[1], Boon Chong Goh[1], Mingzhang Wang[2], Caitlinn Quinn[2], Manman Lu[2], Tatyana Polenova[2], Klaus Schulten[1]

[1]University of Illinois at Urbana-Champaign, Physics and Beckman Institute, Urbana, IL; [2]University of Delaware, Chemistry, Newark, DE

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Capsid

F61. Segmental Labeling of HIV-1 Capsid Protein for Solid State NMR Spectroscopy

Sebanti Gupta and Robert Tycko

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney diseases, National Institutes of Health, MD 20892, USA

F62. Crystal Structures of, P38A, P38A/T216I, E45A and E45A/R132T HIV-1 Capsid Proteins Highlight the Plasticity of HIV-1 Capsid

Anna T. Gres[1,2], Dandan Liu[1,3], Karen A. Kirby[1,3], Qiongying Yang[1,3], Juan R. Perilla[4], Klaus Schulten[4], Jiong Shi[5,6], Christopher Aiken[5], John J. Tanner[2,6], Xiaofeng Fu[7], Peijun Zhang[7], and Stefan G. Sarafianos[1,3,6]

[1]Bond Life Sciences Center, [2]Chemistry, [3]Molecular Microbiology & Immunology, University of Missouri, Columbia, MO; [4]Physics & Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL; [5]Pathology, Microbiology & Immunology, Vanderbilt University, School of Medicine, Nashville, TN; [6]Biochemistry, University of Missouri, Columbia, MO; [7]Structural Biology, University of Pittsburgh, School of Medicine, Pittsburgh, PA

F63. Functionally Important Dynamics in HIV-1 Capsid Assemblies: Atomic-Level Understanding by Integrated MAS NMR, MD, and Density Functional Theory

Huilan Zhang[1,2], Rupal Gupta[1,2], Guangjin Hou[1,2], Manman Lu[1,2], Jinwoo Ahn[2,3], In-Ja Byeon[2,3], Christopher J. Langmead[4], Ivan Hung[5], Peter L. Go'kov[5], Zhehong Gan[5], William Brey[5], Marc Caporini[6], Melanie Rosay[6], Werner Maas[6], Jochem Struppe[6], David A. Case[7], Hartmut Oschkinat[8], Guido Pintacuda[9], Anne Lesage[9], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,2]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 5th Ave., Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA; [4]Computer Science Department, Carnegie Mellon University, 5000 Forbes Avenue, Pittsburgh, PA; [5]National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL; [6]Bruker Biospin Corporation, 15 Fortune Drive, Billerica, MA; [7]BioMaPS Institute and Department of Chemistry and Chemical Biology, Rutgers University, 147 Frelinghuysen Road, Piscataway, NJ; [8]Leibniz-Institut für Molekulare Pharmakologie, Robert-Roessle-Str. 10, 13125 Berlin, Germany; [9]Centre de RMN à Très Hauts Champs, UMR 5280 CNRS / Ecole Normale Supérieure de Lyon, 5 rue de la Doua, 69100 Villeurbanne (Lyon), France

F64. Toward Atomic-Resolution Structure of Conical CA A204C Assemblies by Magic Angle Spinning NMR

Xingyu Lu[1,2], Guangjin Hou[1,2], Huilan Zhang[1,2], Manman Lu[1,2], Mingzhang Wang[1,2], Jinwoo Ahn[2,3], In-Ja L. Byeon[2,3], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,2]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 5th Ave., Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA

F65. Chemical Nature and Physical Properties of the HIV-1 Capsid from All-Atom Molecular Dynamics Simulations

Juan R Perilla and Klaus Schulten

University of Illinois at Urbana-Champaign, Physics and Beckman Institute, Urbana, IL

F66. Virtual Screening of HIV-1 Mature Capsid Protein

Pierrick Craveur, Stefano Forli, and Arthur Olson

Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, California, United States of America

F67. Dynamic Allostery Governs Cyclophilin A - HIV-1 Capsid Interplay

Manman Lu[1,2], Guangjin Hou[1,2], Huilan Zhang[1,2], Christopher L. Suiter[1,2], Jinwoo Ahn[2,3], In-Ja L. Byeon[2,3], Juan R. Perilla[4], Christopher J. Langmead[5], Ivan Hung[6], Peter L. Gor'kov[6], Zhehong Gan[6], William Brey[6], Christopher Aiken[2,7], Peijun Zhang[2,3], Klaus Schulten[4], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,2]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 5th Ave., Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA; [4]Center for Biophysics and Computational Biology and Beckman Institute for Advanced Science and Technology and Departments of Physics, Chemistry, and Biochemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois; [5]School of Computer Science, Computational Biology Department, Carnegie Mellon University, 5000 Forbes Ave., Pittsburgh, PA; [6]National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL; [7]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN

TOC

Membrane Accessory Proteins

F68. Expression and Purification of Pentameric Vpu

Yi-Liang Liu[1], Ignacio Asial[1,2], and Robert M. Stroud[1]

[1]Department of Biochemistry and Biophysics, University of California, San Francisco, California, USA; [2]School of Biological Sciences, Nanyang Technological University, Singapore, Singapore

F69. Arrayed Analysis of Immune Evasion: High Content Imaging Screen Reveals Novel Targets of HIV-1 Vpu

Guney Boso, Prashant Jain, Paul de Jesus, Quy Nguyen and Sumit Chanda

Sanford Burnham Prebys Medical Discovery Institute

F70. Studies of Membrane-Bound HIV Nef to Elucidate its Role in T-Cell Activation

Mathias Lösche[1,2], Michael S. Kent[3], and Thomas E. Smithgall[4]

[1]Dept. of Physics and Dept. of Biomedical Engineering, Carnegie Mellon University; [2]The NIST Center for Neutron Research; [3]Sandia National Laboratories; and [4]Dept. of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine

Influence of 5'-Start Site Heterogeneity and Capping on HIV-1 RNA Structure and Fate

Michael F. Summers[1], Joshua D. Brown[1], Siarhei Kharytonchyk[2], Sarah Monti[1], Thao Tran[1], Steven Choi[1], Michael Lopresti[1], Hannah Carter[1], Aisha Iyer[1], Jana Hiji[1], Nicolas Bolden[1], and Alice Telesnitsky[2]

[1]Howard Hughes Medical Institute and Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250; [2]Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor MI 48109-0620

All lentiviral genomes are transcribed from an integrated proviral DNA that contains a stretch of three sequential guanosines at the U3/R junction, any of which could potentially serve as the transcription start site. We have obtained evidence that the 5´-ends of HIV-1 transcripts produced in infected cells are heterogeneous, and that 5´-end heterogeneity influences RNA fate. Cells infected with HIV-1 produce 5´-capped HIV-1 RNAs that begin with one, two or three guanosines (1G, 2G, and 3G, respectively), with the 1G product selectively packaged into virions and the 2G/3G RNAs enriched on polysomes, apparently preferred for translation. In vitro studies indicate that 5´-end heterogeneity influences the structure of the RNA: Native gel electrophoresis studies indicate that the 5´-capped 1G leader readily forms dimers whereas 5´-capped 2G and 3G RNAs adopt stable monomers. Site directed mutagenesis and ^{2}H-edited NMR studies indicate that the two RNAs adopt significantly different structures and suggest a mechanism that may explain how the presence of a single additional 5´-guanosine can have such a significant influence on structure and fate.

Understanding Translational Regulation in HIV-1

Pham Vincent, Wu Marie Tang-Pei, and D'Souza Victoria

Dept. of Molecular and Cellular Biology, Harvard University

Efficient replication in HIV-1 is dependent upon the maintenance of the ratio between structural (Gag) and enzymatic (Pol) proteins. Pol is expressed exclusively as a Gag-Pol fusion by ribosomal frameshifting. This mechanism occurs infrequently and only affects 5-10% of translating ribosomes, allowing the virus to maintain the critical Gag to Gag-Pol ratio. The frameshifting event requires a *cis*-acting element located downstream of the frameshifting site on the mRNA. In my talk, I will present NMR, SAXS, UV melting and cell-based data that suggest the presence of an alternate conformation that could potentially play a role in determining the frameshifting frequency.

Crystal Structure of the CA-SP1 Assembly and Maturation Switch

Owen Pornillos

University of Virginia

Virus assembly and maturation proceeds through the programmed operation of molecular switches, which trigger both local and global structural rearrangements to produce infectious particles. HIV-1 contains an assembly and maturation switch – the CA-SP1 switch – that spans the C-terminal domain (CTD) of the capsid (CA) region and the first spacer peptide (SP1) of the precursor structural protein, Gag. In collaboration with Barbie Ganser-Pornillos and Mark Yeager at the CHEETAH Center, our group developed ways to crystallize the CTD-SP1 fragment of HIV-1 Gag into its immature lattice form, which is composed of CTD-SP1 hexamers connected by CTD dimers. In the resulting crystal structure, the CTD-SP1 hexamer appears like a goblet, in which the cup comprises the CTD and an ensuing type II beta-turn, and the stem comprises a 6-helix bundle. The 6-helix bundle regulates proteolysis during maturation, and our results indicate that HIV-1 maturation inhibitors prevent unfolding of the CA-SP1 junction and thereby deny access of the viral protease to its substrate. An exciting finding is that nucleation and assembly of the immature Gag lattice appears to be dependent on cooperative folding of both the beta-turn and 6-helix bundle. This supports a model wherein binding of the downstream NC domain to high-affinity sites in the genomic RNA dimer (or an RNA monomer in a dimerization-competent state) promotes folding of the CA-SP1 junction and directly nucleates a Gag hexamer.

Steps in the Assembly of HIV-1

Sanford Simon

Rockefeller University

The biogenesis of HIV-1 was examined from transcription of the genome in the nucleus to scission of the virion at the surface of the cell. Genome dimers were observed in the cytosol. After docking of the genome at the membrane, Gag was observed to be recruited, coincident with the recruitment of 7SL RNA. After Gag recruitment, there was a transient recruitment of ESCRT-III proteins, and, roughly contemporaneously, recruitment of Vps4. Only after ESCRTs and Vps4 could not be detected at the membrane, was there scission of the nascent virion from the cell. Occasionally there were repeated waves of recruitment of ESCRT-III which occurred with repeated waves of Vps4. Once scission occurred, there was no further recruitment of ESCRTs or Vps4.

The HARC Center: Progress and Overview

Yifan Cheng[1,2,3], Charles Craik[2,4], Jennifer Doudna[2,3,5], Alan Frankel[1,2], John Gross[2,4], James Hurley[2,5], Matt Jacobson[2,4], Nevan Krogan[2,6], Matija Peterlin[7], and Robert Stroud[1,2]

[1]Department of Biochemistry and Biophysics, UCSF, San Francisco, CA 94158; [2]California Institute for Quantitative Biosciences, QB3; [3]Howard Hughes Medical Institute, UCSF, San Francisco, CA 94158; [4]Department of Pharmaceutical Chemistry, UCSF, San Francisco, CA 94158; [5]Department of Molecular and Cell Biology, UC Berkeley, Berkeley, CA 94720; [6]Department of Cellular and Molecular Pharmacology, UCSF, San Francisco, CA 94158; [7]Department of Medicine, UCSF School of Medicine, San Francisco, CA 94158

The HARC Center, as part of a large collaborative effort, has developed and applied technological advances towards the goal of achieving comprehensive structural views of HIV-host complexes that are key to essential regulatory and accessory functions. The HIV and host proteins we study are a highly challenging set of targets, often possessing unfolded or dynamic regions that co-fold or adapt to their partners. Themes of plasticity, remodeling and the use of diverse interfaces and partners, as well as evolutionary variability are repeated throughout the projects involving Rev, Vif and Tat complexes. The PR and Vpu projects also present unique challenges related to their activities and environments. Technological approaches to identify and characterize these HIV-human protein complexes, including cryo-EM, mass spectrometry, computational techniques, integrative modeling and Fab-based biochemistry, in addition to genetic approaches such as CRISPR, virus selection and study of human cohort and evolutionary data, are enabling HIV-host complex structure determinations that would not have been possible without this integrated approach. Overall, this work is increasing mechanistic understanding of the virus life cycle and identifying new targets and protein interfaces for intervention as well as expanding the technologies that can be brought to bear on such problems. Through this work, we are achieving a deeper understanding of how the virus subverts the host machinery to control transcription, RNA trafficking, protein degradation, and other essential cellular processes. Updates of several of these ongoing works will be discussed.

Genome Engineering Primary Human T Cells to Test Function of Host Factors in HIV Pathogenesis

Judd F. Hultquist[1,2,3]*, Kathrin Schumann[4]*, Jonathan M. Woo[4,5], Lara Manganaro[6], Jennifer Doudna[5,7,8,9,10], Viviana Simon[6,11,12], Nevan J. Krogan[1,2,3], and Alexander Marson[4,5,13,14]

* *Equal Contribution;* [1]*Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA 94158, USA;* [2]*California Institute for Quantitative Biosciences, QB3, University of California, San Francisco, CA 94158, USA;* [3]*J. David Gladstone Institutes, San Francisco, CA 94158, USA;* [4]*Diabetes Center, University of California, San Francisco, CA 94143, USA;* [5]*Innovative Genomics Initiative, University of California, Berkeley, CA 94720, USA;* [6]*Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA;* [7]*Department of Molecular and Cell Biology, University of California, Berkeley, California;* [8]*Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, California;* [9]*Department of Chemistry, University of California, Berkeley, California;* [10]*Lawrence Berkeley National Laboratory, Physical Biosciences Division, Berkeley, California;* [11]*Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA;* [12]*Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA;* [13]*Division of Infectious Diseases, Department of Medicine, University of California, San Francisco, CA 94143, USA;* [14]*UCSF Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA 94158, USA*

Gene editing in primary human cells has potential to reveal host dependency factors for HIV and other pathogens. We recently developed a method to edit the genome of primary human T cells by electroporation of CRISPR/Cas9 ribonucleoproteins (RNPs). Here, we have adapted this methodology to a high-throughput platform for the efficient, multiplex editing of candidate host factors. Using this platform coupled with *in vitro* HIV infection assays, we disrupted the HIV co-receptors *CXCR4* and *CCR5* to engineer T cells with tropism-dependent blocks to infection. Targeting intra-cellular dependency factors that act after viral entry, including the integration factor *LEDGF* and *TNPO3*, involved in viral export, conferred tropism-independent HIV resistance. We also demonstrated that CRISPR/Cas9 RNPs can disrupt multiple factors simultaneously, providing combinatorial resistance to infection. Ultimately, this strategy can generate HIV-resistant T cells that could contribute to next-generation cell-based therapies aimed at curing HIV infection. Arrayed genome editing in primary T cells will allow unbiased functional studies of host genes encoding proteins that physically interact with HIV proteins. Genetic interaction mapping of host and HIV factors will complement proteomic interaction maps. Looking forward, coupling targeted genome editing in human T cells with genomics and proteomics will enable detailed mechanistic studies of the molecular interactions between host factors and HIV.

Functional Segregation of Overlapping Genes in HIV

Jason D. Fernandes[1,2], Tyler B. Faust[1,3], Nicolas J. Strauli[4,5], Cynthia Smith[1], David C. Crosby[1], Robert Nakamura[1], Ryan D. Hernandez[4], Alan D. Frankel[1]

[1]Department of Biochemistry and Biophysics [2]Program in Pharmaceutical Sciences and Pharmacogenomics [3]Tetrad Program, Department of Biochemistry and Biophysics [4]Department of Bioengineering and Therapeutic Science [5]Biomedical Sciences Graduate Program University of California, San Francisco, CA 94158

Overlapping genes pose an evolutionary dilemma as one region of DNA evolves under the selection pressures of multiple proteins. The degree of constraint that each gene exerts on the other has long remained unclear. To explore this problem, we utilized three complementary datasets to analyze the essential, overlapping HIV-1 genes *tat* and *rev*. First, we analyzed conservation of patient viruses; next, we measured the functional activity of every residue of each protein via alanine scanning; finally, we measured the fitness of engineered, non-overlapped viruses containing every Tat and Rev point mutation. We find a "selfish" organization in which overlapped sites strongly favor a single gene, and where this organization preferentially eliminates unfit genotypes, doubling the proportion of possible fit genotypes. Thus, HIV minimizes the constraint of overlapping genes and redirects that constraint to its own advantage.

Identifying Recombinant Antibodies to Challenging HIV Related Targets

Natalia Sevillano[1], Jennifer Binning[1], Hai Ta[1], Florencia La Greca[1], Judd Hultquist[2,3], Amber Smith[4,5], Nathalie Caretta Cartozo[1], Nevan Krogan[2,3], Yifan Cheng[4,5], John D. Gross[1] and Charles Craik[1]

[1]Department of Pharmaceutical Chemistry, UCSF, San Francisco, CA 94158; [2]Department of Cellular and Molecular Pharmacology, UCSF, San Francisco, CA 94158; [3]J. David Gladstone Institute, San Francisco, CA 94158; [4]Howard Hughes Medical Institute, UCSF, San Francisco, CA 94158; [5]Department of Biochemistry and Biophysics, UCSF, San Francisco, CA 94158

Selective tools for studying HIV-host complexes involved in regulatory and accessory functions is essential for understanding their role in the virus life cycle and for identifying new targets and protein interfaces for intervention. Targeting specific HIV-host complexes for structural studies has been challenging due to the high degree of conformational flexibility that confounds efforts to achieve structural homogeneity. Using the large binding surface on antibodies and their value as diagnostics and therapeutics, we developed phage-displayed, recombinant antibody fragment (Fab) libraries and protocols to identify fully renewable, recombinant antibody-based binders to conformational states of HIV-host proteins and complexes. We have optimized methods to speed the reliable characterization of phage display selected antibody fragments to make the technology more generally applicable. In particular, we have developed a phage display biopanning procedure to identify Fabs for protein complexes so that the Fabs can be rapidly grouped based on relative affinities using enzyme linked immunosorbent assay (ELISA) and unpurified Fabs. This procedure greatly speeds the prioritization of candidate binders to the target and aids in subsequent structure determinations and functional assays. We have recently targeted several HIV-host complexes including the APOBEC3 (A3) family of restriction factors that protect hosts from retroviruses and retroelements. A3 proteins are polynucleotide cytosine deaminases that restrict by mutating viral cDNA. The lentiviral Vif protein promotes spread of virus in the host by targeting A3 family members for degradation by the 26S proteasome. To do so, Vif must hijack an E3 ligase from the Cullin-RING ligase (CRL) superfamily. In primates, the function of the Vif-E3 requires a noncanonical CRL subunit, core-binding factor beta, whereas this requirement is absent or different in non-primate species. Using a reconstituted and characterized HIV-1 Vif substrate receptor complex (Vif-CBFβ-ELOB-ELOC complex (VCBC), we biopanned an Fab phage displayed library to identify high-affinity Fab fragments for the HIV-1 Vif complex to facilitate EM structural studies and functional studies with A3 substrates. In particular, one Fab we identified can inhibit Vif activity *in vitro* and in cells, blocking neutralization of A3F and A3C but not A3G. Our findings document a useful strategy for resolving and inhibiting Vif complexes, and to experimentally establish the feasibility of generating inhibitors that directly target a specific interface of HIV Vif. Agents that inhibit Vif-A3 interactions would restore the restriction potential of A3 family members and be useful as anti-HIV therapeutics. The approach and methodology developed can hopefully be applied to other HIV-host targets and complexes to develop valuable reagents for subsequent studies.

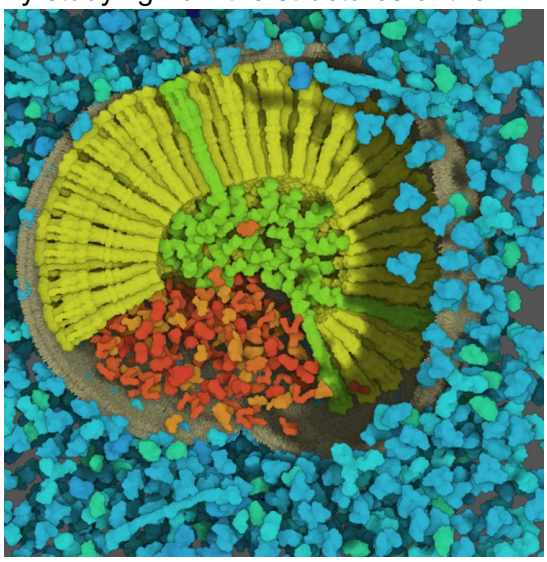
HIVE Center: HIV Interactions and Viral Evolution of Drug Resistance

Eddy Arnold[1], John Elder[2], Alan Engelman[3], M.G. Finn[4], Barry Sharples[5], Patrick Griffin[6], Stephen Hughes[7], Roger Jones[1], Mamuka, Kvaratskhelia[8], Ron Levy[1], Joe Marcotrigiano[1], Arthur Olson[9], Stephanos Sarafianos[10], Alan Rein[7], Dave Stout[9], Bruce Torbett[11], Karin Musier-Forsyth[12], Dymitri Lyumkis[13], and Jeffrey DeStephano[14]

[1]Department of Chemistry and Chemical Biology, Rutgers University; [2]Department of Immunology and Microbial Science, The Scripps Research Institute; [3]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute; [4]Department of Chemistry and Biochemistry, Georgia Institute of Technology; [5]Department of Chemistry, The Scripps Research Institute; [6]Department of Molecular Therapeutics The Scripps Research Institute Florida; [7]Center for Cancer Research, National Cancer Institute, NIH; [8]College of Pharmacy, The Ohio State University; [9]Department of Integrative Structural and Computational Biology, The Scripps Research Institute; [10]Department of Microbiology & Molecular Genetics, University of Missouri; [11]Department of Molecular and Experimental Medicine, The Scripps Research Institute; [12]Department of Chemistry, The Ohio State University; [13]The Salk Institute; [14]Department of Cell Biology and Molecular Genetics, University of Maryland

The HIVE Center is focused on the structural and dynamic interactions of the major HIV enzymes, reverse transcriptase/RNAse H, protease and integrase, with their molecular partners and effectors in key processes of the viral life cycle. By studying how the structures of the HIV polyprotein precursors direct assembly, maturation and replication, as well as how HIV-Host interactions drive DNA replication and integration, we explore how therapeutic targeting impacts the evolution of drug resistance and what the structural and dynamic consequences of resistance mutations are on the HIV life cycle. This approach is significant because of the promise of new structural insights into the interdependence of viral mechanisms and the potential for new drug design methodologies and therapeutic strategies.

This overview will present the nature of the collaborations and research within the HIVE Center, with selected examples of work that highlight the connections between experimental and computational approaches to understanding HIV structures and the evolution of drug resistance.



CellVIEW image of CellPACK model, immature HIV Virion

HIV-1 Integrase Binds the Viral RNA Genome and is Essential During Virion Morphogenesis

Jacques J. Kessl[1,2,3*], Sebla B. Kutluay[4,5*], Dana Townsend[5], Stephanie Rebensburg[2,3], Alison Slaughter[1,3], Ross C. Larue[2,3], Nikoloz Shkriabai[2,3], Nordine Bakouche[2,3], James R. Fuchs[1,3], Paul D. Bieniasz[4,6,7], Mamuka Kvaratskhelia[1,2,3]

*[1]HIVE Center, [2]Center for Retrovirus Research, [3]College of Pharmacy, The Ohio State University, Columbus, OH 43210; [4]CRNA Center, [5]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110; [6]Laboratory of Retrovirology, [7]Howard Hughes Medical Institute, Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY 10016, USA; *These authors contributed equally*

While an essential role of HIV-1 integrase (IN) for integration of viral cDNA into human chromosome is established, studies with IN mutants and allosteric IN inhibitors (ALLINIs) have suggested that IN can also influence viral particle maturation. However, it has remained enigmatic as to how IN contributes to virion morphogenesis. Using crosslinking-immunoprecipitation sequencing we have uncovered that IN binds the viral RNA genome in virions. These interactions have specificity as IN exhibits distinct preference for select viral RNA structural elements. We show that IN substitutions that selectively impair its binding to viral RNA result in eccentric, non-infectious virions without affecting nucleocapsid-RNA interactions. Likewise, ALLINIs impair IN binding to viral RNA in virions of wild type but not escape mutant virus. These results reveal an unexpected biological role of IN binding to the viral RNA genome during virion morphogenesis and elucidate the mode of action of ALLINIs.

A New Class of Allosteric HIV-1 Integrase Inhibitors Identified by Crystallographic Fragment Screening of the Catalytic Core Domain

Disha Patel,[1] Janet Addae-Antwi,[2] Pratibha C. Koneru,[3] Erik Serrao,[4] Stefano Forli,[5] Jacques Kessl,[3] Lei Feng,[3] Nanjie Deng,[6] Ronald M. Levy,[6] James R. Fuchs,[2] Arthur J. Olson,[5] Alan N. Engelman,[4] Joseph D. Bauman,[1] Mamuka Kvaratskhelia,[3] and Eddy Arnold[1]

[1]Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ; [2]Division of Medicinal Chemistry and Pharmacognosy, The Ohio State University, Columbus, OH; [3]Center for Retrovirus Research and College of Pharmacy, The Ohio State University, Columbus, OH; [4]Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute and Department of Medicine, Harvard Medical School, Boston, MA; [5]Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA; [6]Center for Biophysics and Computational Biology, Temple University, Philadelphia , PA

HIV-1 integrase (IN) is essential for virus replication and represents an important multifunctional therapeutic target. Recently discovered quinoline-based allosteric IN inhibitors (ALLINIs) potently impair HIV-1 replication and are currently in clinical trials. ALLINIs exhibit a multimodal mechanism of action by inducing aberrant IN multimerization during virion morphogenesis and competing with IN for binding to its cognate cellular cofactor LEDGF/p75 during early steps of HIV-1 infection. However, quinoline-based ALLINIs have a low genetic barrier for the selection of resistant phenotypes, which highlights a need for discovery of second generation inhibitors. Using crystallographic screening of a library of 971 fragments against the HIV-1 IN catalytic core domain (CCD) followed by a fragment expansion approach, we have identified thiophenecarboxylic acid-derivatives that bind at the CCD-CCD dimer interface at the principal LEDGF/p75 binding pocket. The most active fragment inhibited LEDGF/p75-dependent HIV-1 IN activity *in vitro* with an IC_{50} of 72 μM and impaired HIV-1 infection of T cells at an EC_{50} of 36 μM. These findings are significant because the identified lead fragment with a relatively small molecular weight (221 Da) provides an optimal building block for developing a new class of inhibitors. Furthermore, while structurally distinct thiophenecarboxylic acid-derivatives target a similar pocket at the IN dimer interface as the quinoline-based ALLINIs, the lead fragment **5** inhibited IN mutants that confer resistance to quinoline-based compounds. Collectively, our findings provide a plausible path for structure-guided development of second generation ALLINIs.

Crystal Structure of Prototype Foamy Virus PR-RT Polyprotein Shows Unique Architecture Among Retroviruses: Implications for Function

Jerry Joe Harrison,[1] Steve Tuske,[1] Kalyan Das,[1] Joseph D. Bauman,[1] Paul L. Boyer,[2] Stephen H. Hughes,[2] and Eddy Arnold[1]

[1]Center for Advanced Biotechnology and Medicine, and Departments of Chemistry and Chemical Biology, and Medicinal Chemistry, Rutgers University, Piscataway, NJ; [2]HIV Drug Resistance Program, National Cancer Institute, Frederick, MD

Retroviruses conventionally synthesize their proteins from polycistronic mRNAs into polyproteins, and the polyproteins are proteolytically processed by the viral proteases into functional entities. We have engaged in systematic structural study of the Pol polyprotein of prototypic foamy virus (PFV), a surrogate of HIV. Here, we report the crystal structure of the protease-reverse transcriptase (PR-RT) polyprotein of PFV that functions as the viral transcription and processing machinery. While the monomeric PFV PR exhibits similar architecture as the HIV-1 PR, it contains a C-terminal extension containing two helices, associated with the equivalent of HIV-1 RT helices E and F beneath the base of the palm subdomain. The PR is anchored to the RT adjacent to the fingers and palm subdomains by these two helices via a long loop. This arrangement allows the PR to swing open to enable dimerization without disturbing the integrity of the RT; like in HIV, PFV PR is active as a dimer. The structural components (the fingers, palm, and thumb subdomains) are highly conserved in all retroviruses and retrotransposon RTs that carry out the addition of deoxynucleotides to a DNA strand using a common catalytic mechanism. The organization of the polymerase (fingers, palm, thumb, connection, and RNase H) in PFV PR-RT is significantly different when compared to other retroviral polymerases. Three functional entities PR, RT, and RNase H are connected by flexible linkers. Notably, the thumb C-terminus and RNase H N-terminus are connected to the connection subdomain via long flexible linkers that permit spatial rearrangement of the subdomains. The novel spatial arrangements of the thumb, connection, and RNase H in the current structure suggest that the PFV PR-RT would undergo significant conformational rearrangements upon nucleic acid binding. This structure opens up the frontiers for modeling the polyproteins of other retroviruses such as HIV-1 to enhance our understanding of retroviral polyprotein processing.

Visualization of Viral RNA and DNA Dynamics During HIV Infection

Maritza Puray-Chavez[1,2], Andrew D. Huber[1,3], Dandan Liu[1,2], Obiaara Ukah[1,2], Vincent Yapo[1,2], Juan Ji[1], Jennifer J. Wolf[1,2], Gregory A. Sowd[4], Alan N. Engelman[4] and Stefan G. Sarafianos[1,2,3,5]

[1]Christopher S. Bond Life Sciences Center, [2]Molecular Microbiology & Immunology, [3]Veterinary Pathobiology, University of Missouri, Columbia, MO; [4]Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, Boston, MA; [5]Biochemistry, University of Missouri, Columbia, MO

To better understand the HIV life cycle, we developed a fluorescence microscopy approach which utilizes branched DNA *in situ* hybridization technology on fixed cells to visualize single copies of viral RNA (vRNA) and DNA (vDNA). Viral nucleic acids were observed following infection at relatively low multiplicity of infection (MOI, typically 0.5) in semi-synchronized or synchronized cells. Target cells included HeLa-derived TZM-bl, lymphocytes (H9, Jurkat, MT-2), monocytes (THP-1), HEK293T, and primary cells.

Protocols were developed to simultaneously monitor in individual fixed cells unspliced and spliced+unspliced vRNA, negative sense vDNA, and integrated proviral DNA. Specificity was confirmed a) by the absence of fluorescence signal in uninfected cells, b) susceptibility of the vRNA, but not of the vDNA fluorescence signal to RNase A treatment of the samples prior to hybridization, and c) susceptibility of the vDNA, but not of the vRNA fluorescence signal to DNase I treatment prior to hybridization. Specific detection of HIV-1 and HIV-2 enabled visualization of HIV-1/HIV-2 co-infection events in individual cells.

Reverse transcription in TZM-bl cells was generally completed by ~10 hours post infection (hpi) as judged by the gradual disappearance of vRNA signal, presumably due to RNase H activity, with concomitant increase of nascent vDNA in the cytoplasm. vRNA signal was rescued in the presence of an RNase H inhibitor. We commonly observed co-localization of HIV capsid (CA) with vRNA and nascent vDNA that were presumably encapsidated. Both cytoplasmic and proviral DNA signals disappeared upon EFdA, or AZT treatment, whereas raltegravir specifically suppressed proviral/nuclear, but not cytoplasmic vDNA.

At low MOIs, we typically observed one or two integration sites. Spliced vRNA was observed exported from the nucleus prior to unspliced vRNA. Unspliced RNA transcripts began to appear at ~12 hpi, centered at individual transcription sites and at times appearing as transcriptional "bursts" co-localized with integration sites. Notably, not all integrated DNA sites appeared transcriptionally active. Large amounts of nascent unspliced RNA transcripts accumulated in the nucleus until ~14-16 hpi, presumably when Rev enabled their highly efficient export from the nucleus. CA protein also appeared in the cytoplasm at ~14-16 hpi. At ~24 hpi, new virions appeared to infect nearby cells. We have expanded this approach to monitor distribution of vRNA and vDNA upon knockdown or knockout of host factors, including CPSF6, LEDGF/p75, CypA, or MOV10, or upon treatment with additional antivirals targeting reverse transcriptase, integrase, or CA.

Dynamic Regulation of HIV-1 Capsid Assembly, Maturation, and Interactions with Host Factors by Integrated MAS NMR and MD Simulations

Manman Lu[1,2], Huilan Zhang[1,2], Guangjin Hou[1,2], Rupal Gupta[1,2], Caitlin Quinn[1,2], Mingzhang Wang[1,2], Jinwoo Ahn[2,3], In-Ja Byeon[2,3], Sherimay Ablan[4], Juan R. Perilla[5], Christopher J. Langmead[6], David A. Case[7], Christopher Aiken[2,8], Eric O. Freed[4], Peijun Zhang[2,3], Klaus Schulten[5], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,2]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 Fifth Ave., Pittsburgh, PA 15261; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA 15261; [4]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702; [5]University of Illinois, Theoretical and Computational Biophysics Group, Urbana, IL 61801; [6]Computer Science Department, Carnegie Mellon University, Gates Hillman Center, 5000 Forbes Avenue, Pittsburgh, PA; [7]BioMaPS Institute and Department of Chemistry and Chemical Biology, Rutgers University, 147 Frelinghuysen Road, Piscataway, NJ 08854-8087; [8]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN 37232

HIV-1 capsids, assembled from ~1,500 copies of the capsid (CA) protein, are an integral part of mature virions [1, 2]. Conical in shape, capsids enclose the viral genetic material (two copies of RNA) together with several proteins that are essential for viral replication. In the assembled state, capsids are remarkably dynamic, with the CA residue motions occurring over a range of timescales from nano- to milliseconds [3-6]. These motions are functionally important for capsid's assembly, viral maturation, and interactions with host factors [3, 6]. In this talk, I will discuss the recent progress in atomic-resolution structural and dynamics studies of CA assemblies by magic angle spinning (MAS) NMR spectroscopy. I will present an integrated MAS NMR and MD simulations approach to probe functionally important motions in assemblies of CA and their complexes with host factor Cyclophilin A (CypA), as well as assemblies of CA-SP1 maturation intermediates. I will discuss the role of dynamic allosteric regulation in capsid's assembly, maturation, and escape from the CypA dependence. Our studies demonstrate that the integration of experimental NMR methods and theory yields quantitative, atomic-level insights into the dynamic processes that govern the HIV-1 capsid's function.

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HIV-1 and HIV-2 Exhibit Divergent Interactions with DNA Repair Enzymes

Jacek Skowronski

Case Western Reserve School of Medicine, Cleveland, OH

In non-dividing host cells HIV is targeted by intrinsic anti-viral defense mechanisms that introduce marks of damage into viral cDNA, thereby tagging it for processing by the cellular DNA repair machinery whose role(s) in HIV replication cycle is not well understood. HIV-1 virion-associated accessory virulence factor Vpr hijacks $CRL4^{DCAF1}$ E3 ubiquitin ligase and coordinates interactions with the postreplication DNA repair machinery, but the extent of its interactions with DNA repair proteins hasn't been thoroughly characterized. Here, using proteomics and functional assays we identify HLTF, a postreplication DNA repair helicase, as a new common target of HIV-1/SIVcpz Vpr proteins. We show that HIV-1 Vpr reprograms $CRL4^{DCAF1}$ E3 to direct HLTF for proteasome-dependent degradation, in a manner that is independent from previously reported Vpr interactions with base excision repair enzyme UNG2 and crossover junction endonuclease MUS81, which Vpr also directs for degradation via $CRL4^{DCAF1}$ E3. Thus, separate functions of HIV-1 Vpr usurp $CRL4^{DCAF1}$ E3 to remove key enzymes in three distinct DNA repair pathways. In contrast, we find that HIV-2 Vpr is unable to efficiently program HLTF or UNG2 for degradation. Our findings reveal complex interactions between HIV-1 and the DNA repair machinery, suggesting that DNA repair plays important roles in HIV-1 life cycle. The divergent interactions of HIV-1 and HIV-2 with DNA repair enzymes imply that these viruses use different strategies to guard their DNA genomes and/or facilitate their replication in the host.

To Repair or Not Repair: The X-Ray Crystal Structure of the DDB1-DCAF1-Vpr-UNG2 Complex

Guillermo Calero, Ying Wu, Xiaohong Zhou, Christopher O. Barnes, Maria DeLucia, Aina E. Cohen, Angela M. Gronenborn, Jinwoo Ahn

University of Pittsburgh, Pittsburgh, Pennsylvannia

The HIV-1 accessory protein Vpr is required for efficient viral infection of macrophages and promotion of viral replication in T-cells. The biological activities of Vpr are closely tied to the interaction with DCAF1, a cellular substrate receptor of the Cullin4-RING E3 ubiquitin ligase (CRL4) of the host ubiquitin proteasome-mediated protein degradation pathway. At present, the molecular details of how Vpr usurps the protein degradation pathway have not been delineated. Here, we present the crystal structure of the DDB1-DCAF1-HIV-1-Vpr-Uracil-DNA glycosylase (UNG2) complex. The structure reveals how Vpr engages with DCAF1 and creates the special binding interface for UNG2 recruitment, distinct from how the related Vpx proteins recruit SAMHD1 for degradation. Vpr and Vpx utilize similar N-terminal and helical regions to bind the substrate receptor, while distinctly different regions target the specific cellular substrates. Furthermore, Vpr employs molecular mimicry of DNA by a variable loop for specific recruitment of the UNG2 substrate.

BACK

Introduction to the CHEETAH Center

Wes Sundquist

Department of Biochemistry, University of Utah

D-Peptide Fusion Inhibitor Protects Against High-Dose SHIV Challenge

Michael S. Kay

Department of Biochemistry, University of Utah School of Medicine

Peptides excel at disrupting “undruggable” protein-protein interactions, but their in vivo fragility hampers their therapeutic potential. Our lab develops mirror-image D-peptide inhibitors that resist proteases and enjoy extended half-lives. We employ high-throughput mirror-image phage display screening and structure-based design to identify D-peptides that block viral entry. Our anti-HIV D-peptide (cholesterol-conjugated PIE12-trimer) is a pM inhibitor of viral entry and is highly active against in the macaque SHIV infection model for both prevention and treatment. This D-pepitde is now in advanced preclinical development and is ideally suited for sustained-release formulation.

Visualizing HIV Uncoating in Living Cells

Thomas J. Hope

Departments of Cell and Molecular Biology, OB/GYN, and Biomedical Engineering, Northwestern University

Individual viral particle tracking and analysis has become widely utilized as a tool to study the cell biology of the early steps of HIV replication. Although this approach has advanced the field, it has faced the important criticism that it is not possible to filter the observations to focus on the particles that ultimately infect a cell. Utilizing variable time lapse imaging of multiple fields of cells over periods up to 36 hours allows the identification of productively infected cells as visualized by reporter virus expression. Infection with very low MOI allows conditions where there is less than one labeled particle per cell. In this way correlation of individual particle behavior can be directly connected to infection. We have recently developed the use of intravirion fluid phase markers to determine the integrity of the HIV conical capsid core. To visualize dynamic changes in capsid integrity and composition, we utilized the HIV-iGFP construct. During viral maturation of HIV-iGFP, the GFP is liberated from Gag; a minority population of the free GFP is trapped in the capsid, while the remaining free GFP is located outside of the capsid. With this technique, the loss of the fluid phase GFP occurs in two steps, with fusion and upon the loss of capsid core integrity. Live-cell microscopy of HIV-iGFP virions with a second color labeled viral core protein such as Vpr or Integrase allows for the precise timing of these two steps thereby revealing the kinetics, localization and composition of the early steps of HIV-1 infection. Our studies reveal that the time between fusion and capsid integrity loss, for both HIV and VSV-G mediated fusion, in tissue culture and primary cells (macrophages and T cells), is approximately 25 minutes. Also, capsid integrity loss occurs entirely in the cytoplasm. However, we also detect a second species, which does not experience a change in capsid integrity over 2 hours of imaging. Through viral challenge with less than one particle per cell over a long period of time, we were able to image individual particle capsid integrity loss that produces a viable infection. This analysis revealed that all particles associated with cellular infection showed changes in capsid integrity ~25 minutes. Together, these observations validate the early cytoplasmic uncoating model.

Structural Studies of TRIM5α Assembly

Barbie K. Ganser-Pornillos

Dept. of Physiology and Biological Physics, University of Virginia

TRIM5α is a restriction factor that recognizes the incoming capsids of retroviruses. Although the exact mechanism of restriction is still poorly understood, capsid recognition leads to accelerated capsid dissociation, disrupted reverse transcription, and enhanced interferon signaling.

TRIM5α is a member of a large family of proteins that contain a conserved RBCC motif consisting of a RING domain, one or two B-box domains, and a coiled-coil domain. TRIM5α also has a C-terminal SPRY domain that directly binds the viral capsid. Efficient capsid recognition requires higher-order assembly of TRIM5α, which is mediated by the RBCC motif. We have previously shown that TRIM5-21R, an artificial rhesus TRIM5 chimera that contains the RING domain of human TRIM21, spontaneously assembles into large hexagonal nets. Assembly of the TRIM5-21R hexagonal net can be induced *in vitro* by mimics of the HIV-1 capsid. We have therefore proposed that complementarity between the TRIM nets and underlying CA hexagonal lattice drives avid capsid recognition.

We now report that hexagonal lattice assembly is a conserved property of TRIM5α homologs from different mammalian species. 2D hexagonal arrays of HIV-1 CA can induce assembly of HIV-restrictive TRIM5α isoforms, but not non-restrictive isoforms. Imaging and analysis by cryoEM and cryoET revealed that the hexagonal TRIM lattice wraps around the curved CA lattices in HIV-1 CA tubes and purified cores. The SPRY domain was localized by cryoET analysis to the 2-fold symmetric hexagon edges in the TRIM lattice, and a combination of crystallographic, biochemical, and virological experiments demonstrated that the 3-fold symmetric vertices consist of B-box 2 domain trimers. Finally, we suggest how the lattice achieves the structural flexibility necessary for TRIM5 to accommodate the variable curvature and pleomorphism of retroviral capsids. Thus, our studies have revealed important principles that explain how TRIM5 proteins recognize retroviral capsid.

Structure and Function of RNA and Proteins from Natural Sequence Variation

Debora S. Marks

Systems Biology & CBMI, Raymond and Beverly Sackler Laboratory for Computational Biology, Harvard Medical School

Modern genome sequencing and synthesis can acquire and generate tremendous molecular diversity in a day, but our ability to navigate and interpret the exponentially large space of possible biological sequences remains limited. We mine the evolutionary sequence record to derive precise information about the function and structure of RNAs and RNA-protein complexes. As in protein structure prediction, we use maximum entropy global probability models of sequence co-variation to infer evolutionarily constrained nucleotide-nucleotide interactions within RNA molecules and nucleotide-amino acid interactions in RNA-protein complexes. The predicted contacts allow all-atom blinded 3D structure prediction at good accuracy for several known RNA structures and RNA-protein complexes. For unknown structures, we predict contacts in 160 non-coding RNA families. Beyond 3D structure prediction, evolutionary couplings help identify important functional interactions—e.g., at switch points in riboswitches and at a complex nucleation site in HIV. Aided by increasing sequence accumulation, evolutionary coupling analysis can accelerate the discovery of functional interactions and 3D structures involving RNA.

Structural Basis for Inhibitor-Induced Aggregation of HIV Integrase

Kushol Gupta[2*], Vesa Turkki[1*], Scott Sherrill-Mix[1], Young Hwang[1], Grant Eilers[1], Louis Taylor, Charlene McDanal[4], Ping Wang[4], David Temelkoff[4], Robert T. Nolte[3], Emile Velthuisen[4], Jerry Jeffrey[4], Gregory D. Van Duyne[2+] and Frederic D. Bushman[1+]

*[1]From the Perelman School of Medicine, University of Pennsylvania. Department of Microbiology 3610 Hamilton Walk Philadelphia, PA 19104-6076 U.S.A.; [2]Department of Biochemistry and Biophysics 242 Anatomy-Chemistry Building Philadelphia, PA, 19104-6059 U.S.A.; [3]Protein Cellular and Structural Science GlaxoSmithKline Collegeville, PA 19426 U.S.A.; [4]HIV DPU Infectious Disease Therapy Area Unit GlaxoSmithKline RTP, NC 27709 U.S.A.; *these authors contributed equally; +corresponding authors*

The allosteric integrase (IN) inhibitors (ALLINIs) target the viral-encoded IN protein and interfere with HIV replication via disruption of viral particle assembly late during HIV replication. To investigate their inhibitory mechanism, we crystallized full-length HIV-1 IN bound to the ALLINI GSK1264, and determined the structure of the complete inhibitor interface at 4.4 Å resolution. In this structure, GSK1264 is buried between the catalytic core domain of one IN dimer and the CTD of an adjacent IN dimer. The amino-terminal domain is not resolved in the structure but does not participate in GSK1264 binding. The GSK1264 binding interface is rich in residues implicated in IN oligomerization and ALLINI sensitivity, indicating likely functional significance. The IN-IN interaction mediated by GSK1264 leads to formation of an open polymer in the crystal, a polymerization reaction that is readily reproduced in solution with purified components. To probe ALLINI function more broadly, we compared the properties of several ALLINIs in biochemical, virological, and electron microscopic assays. Several ALLINI escape mutations encode IN substitutions at or near the inhibitor binding site, and these also resulted in decreased IN oligomerization *in vitro*. The results support a mechanism where ALLINIs disrupt viral particle maturation by promoting formation of the IN polymers observed in the IN-GSK1264 crystal structure. Additionally, the results support a structural model for the catalytically inactive IN tetramer discussed in several previous studies. Identification of the interface responsible for polymer formation provides data useful for improving HIV inhibitors and helps explain a wealth of previous studies of HIV IN.

Biochemical Reconstitution of Selective HIV-1 Genome Packaging

Lars-Anders Carlson[1], Yun Bai[1,3], Sarah C. Keane[2], Jennifer A. Doudna[1], and James H. Hurley[1]

[1]Department of Molecular and Cell Biology, University of California, Berkeley; [2]University of Maryland, Baltimore County; [3]Current address: School of Life Science and Technology, ShanghaiTech University, Shanghai, 201210, China

HIV-1 Gag selectively packages the genomic viral RNA into assembling virus particles in the context of a large excess of cytosolic human RNAs. As Gag assembles on the plasma membrane, the HIV-1 genome is enriched relative to cellular RNAs by a mechanism which is incompletely understood. We used a minimal system consisting of purified RNAs, recombinant myristylated HIV-1 Gag and giant unilamellar vesicles to recapitulate the selective packaging of the 5' untranslated region of the HIV-1 genome in the presence of excess competitor RNA. In this assay, the Rev-responsive element and a non-viral control RNA were not selected for to the same degree. Mutations in the CA-CTD domain of Gag which subtly affect the self-assembly of Gag on membranes abrogated RNA selectivity. We further found that tRNA suppresses Gag membrane binding less when Gag has bound viral RNA. The ability of HIV-1 Gag to selectively package its RNA genome and its self-assembly on membranes are thus interdependent on one another.

Structure of a Natively-Glycosylated HIV-1 Env Reveals a New Mode for VH1-2 Antibody Recognition of the CD4 Binding Site Relevant to Vaccine Design

Harry B. Gristick[1]*, Lotta von Boehmer[2]*, Anthony P. West Jr.[1], Michael Schamber[1], Michel C. Nussenzweig[2,3], and Pamela J. Bjorkman[1]

*[1]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA; [2]Laboratory of Molecular Immunology and [3]Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10065, USA; *These authors contributed equally.*

The trimeric HIV-1 envelope glycoprotein (Env), the only target of neutralizing antibodies on HIV-1, includes glycans constituting up to 50% of its mass attached to 30±3 potential *N*-linked glycosylation sites (PNGSs) per gp120-gp41 protomer. Viral glycans are generally non-immunogenic because they are assembled by host cell machinery, thus the carbohydrates decorating the surface of Env constitute a "glycan shield" that reduces access to underlying immunogenic protein epitopes. Structural studies of broadly neutralizing antibodies (bNAbs) bound to Env trimers have revealed mechanisms by which bNAbs targeting various epitopes penetrate the glycan shield to either accommodate or include *N*-glycans in their epitopes. Although accessibility to the conserved host receptor (CD4) binding site (CD4bs) is restricted by surrounding glycans, VRC01-class bNAbs mimic CD4 binding to share a common mode of gp120 binding and glycan accommodation using a VH1-2*02-derived variable heavy (V_H) domain. While attractive candidates for immunogen design, features of VRC01-class bNAbs such as a high degree of somatic hypermutation (SHM) and a short (5-residue) light chain (LC) complementarity determining region 3 (CDRL3) (found in only 1% of human LCs) suggest they might be difficult to elicit through vaccination. Here we present a crystal structure of a fully- and natively-glycosylated Env trimer, which we use to define the complete epitopes of IOMA, a new class of CD4-mimetic bNAb, and the V3-loop-directed bNAb 10-1074. Although derived from VH1-2*02, IOMA has a normal-length CDRL3 and includes fewer SHMs than VRC01-class bNAbs, suggesting it may be easier to elicit than VRC01-class bNAbs. Analysis of the native glycan shield on HIV-1 Env allows the first full description of the interplay between heterogeneous untrimmed high-mannose and complex-type *N*-glycans within the CD4bs, V3-loop, and other epitopes on Env, revealing antibody-vulnerable glycan holes and roles of complex-type *N*-glycans on Env that are relevant to vaccine design.

Overview and Progress Summary for P01GM056550: Structure-Based Antagonism of HIV-1 Envelope Function in Cell Entry

Irwin Chaiken[1], Cameron Abrams[1], Ernesto Freire[2], Wayne Hendrickson[3], Walther Mothes[4], Joseph Sodroski[5] and Amos B. Smith III[6]

[1]Drexel University; [2]Johns Hopkins University; [3]Columbia University; [4]Yale University; [5]Harvard University; [6]University of Pennsylvania

Our Program employs a synergistic approach to investigate strategies for inhibiting HIV-1 gp120 in the metastable virus Env protein trimer. We identify and develop gp120 inhibitory compositions, and at the same time use inhibitors to define fundamental concepts, mechanisms and targetable vulnerabilities in Env. It is now well established that Env can traverse an energetically favorable cascade of host cell CD4 and co-receptor interactions that in turn integrate conformational rearrangements leading to virus-cell and cell-cell membrane fusion, entry and infection. A hallmark achievement of the Program has been to identify stages along this "activation trajectory" at which different classes of molecules can block progression to entry. A prime example has been the development of small molecule CD4 mimetics (SMCMs) that, while minimizing enhancement of infection in CD4-negative cells, are able to sensitize Env gp120 for neutralizing antibody binding, with promising consequences for prevention and treatment. At the same time, Env inhibitors have been identified that can divert the conformationally dynamic Env trimer towards an "inactivation trajectory". A recently developed path of work has led to macrocyclic peptide triazoles (cPTs) that dual antagonize gp120 receptor binding sites, disrupt Env trimer assembly before cell encounter and inactivate virus, including with antagonist variants that appear to trigger Env transformations similar to those found in the activation trajectory. The thermodynamic behavior of SMCMs and cPTs are unique and reflect the different conformational transformation pathways used. The binding sites for inhibitors and inactivators are being investigated by a multi-disciplinary approach including crystallography, computation, mutagenesis and patterns of virus resistance. Intriguingly, SMCMs and cPTs share overlapping binding sites, including in the context of emerging trimer structures, yet with different functional outcomes. The structural mechanisms of binding are being used to advance inhibitor designs. Overall, these efforts are expanding our understanding of both the structural and dynamic mechanisms of the HIV-1 Env protein entry machine, and molecular strategies available to hijack the machinery built into the Env protein for potential use in disease intervention and prevention.

Conformational Transformations Triggered by Different Inhibitor Classes

Arne Schön and Ernesto Freire

Johns Hopkins University

Cell infection by HIV-1 requires the activation of the envelope glycoprotein of HIV-1 into an infection competent state. At the molecular level, this process involves binding of gp120 to the cell surface receptor CD4, and a conformational change triggered by that same binding event. The nature of this conformational change has been examined thermodynamically and characterized as a process involving the folding of intrinsically disordered domains. The complex nature of the mechanism of action of gp120 suggests different strategies to inhibit cell entry, including competitive inhibition and/or trapping gp120 into an unproductive conformation. In this Program Project, competitive and non-competitive inhibitors have been identified and their antiviral potency is being optimized. The coupling between binding and conformational equilibrium requires not only optimization of the binding affinity, but of the conformational effects of binding. In the case of competitive inhibitors (small molecule CD4 mimetics) it is required that the inhibitors do not become agonists and trigger the same changes as CD4. In the case of non-competitive inhibitors (peptide triazoles and macrocyclic peptide triazoles) it is necessary to drive gp120 into an unproductive conformation with as much free energy as possible. Each class of inhibitor is characterized by a distinct thermodynamic signature that serves as a blueprint for optimization. In this presentation, the thermodynamic characteristics of both classes of inhibitors will be discussed.

An Intermediate State of the HIV-1 Envelope Glycoproteins on the Entry Pathway

Alon Herschhorn[1,2], Xiaochu Ma[3], Christopher Gu[1], John D. Ventura[3], Bruno Melillo[4], Daniel S. Terry[5], Amos B. Smith III[4], Scott C. Blanchard[5], James B. Munro[6], Walther Mothes[3], Andrés Finzi[7] and Joseph Sodroski[1,2,8]

[1]Department of Immunology Cancer and Virology, Dana-Farber Cancer Institute, Boston, MA 02215, USA; [2]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA 02215, USA; [3]Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06536, USA; [4]Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, USA; [5]Department of Physiology and Biophysics, Weill Cornell Medical College of Cornell University, New York, NY 10021, USA; [6]Department of Molecular Biology and Microbiology, Tufts University School of Medicine and Sackler School of Graduate Biomedical Sciences, Boston, MA 02110, USA;[7]Centre de Recherche du CHUM and Department of Microbiology, Infectiology and Immunology Université de Montréal, Montreal, Quebec, Canada; Department of Microbiology and Immunology, McGill University, Montreal, Quebec, Canada; [8]Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health, Boston, MA 02115, USA

Primary human immunodeficiency virus (HIV-1) envelope glycoprotein (Env) trimers $(gp120/gp41)_3$ typically exist in a metastable closed conformation (State 1). Binding the CD4 receptor triggers Env to undergo extensive conformational changes to mediate virus entry. We identified specific gp120 residues that control HIV-1 Env conformation. Changes in these residues regulate transitions to downstream conformations and allow the Env to sample a functional conformation (State 2) intermediate between State 1 and the full CD4-bound state (State 3). State 2 was an obligate intermediate for all transitions between State 1 and State 3 and increased State 2 occupancy was associated with lower energy barriers between the states. State-2-enriched Envs required lower CD4 concentrations to trigger virus entry and exhibited an improved ability to infect primary macrophages. These Envs were resistant to several broadly neutralizing antibodies and small-molecule inhibitors. Thus, State 2 is an Env conformation on the virus entry pathway; frequent sampling of State 2 increases the adaptability of HIV-1 to different host cell receptor levels and immune environments. Our results provide new insights into the conformational regulation of HIV-1 entry.

Project Overview - The Interdependency of Drug Resistance Evolution and Drug Design: HIV-1 Protease, a Case Study

R. Swanstrom[2], W. Sherman[3], A. Ali[1], K. Zeldovich[1], N. Kurt-Yilmaz[1], S Lee[2], R. Ishima[4], J. Luban[1], D. Bolon[1], C. Schiffer[1]

[1]UMass Medical School, Worcester, MA; [2]University of North Carolina School of Medicine, Chapel Hill, NC; [3]Schrodinger, Inc.; [4]School of Medicine, University of Pittsburgh

In this project we are elucidating the underlying interdependencies under which drug resistance is selected using HIV-1 protease as a model system. Drug resistance occurs when the balance favors viral replication between proteolytic activity within the virus and enzyme inhibition. We combine analysis of sequence diversity, structure, dynamics and energetics to elucidate the underlying pathways and additional macromolecular pressures that contribute to drug resistance.

In this year we have probed the interdependency of resistant variants that arose from viral passaging, through a combination of structural, dynamic and mutational methods. In each case we bridge experimental and computational techniques and are developing a database where multiple data sets can be probed to elucidate their interdependencies.

Establish a Strategy to Elucidate Molecular Mechanisms Leading to Drug Resistance

R. Swanstrom[2], W. Sherman[3], A. Ali[1], K. Zeldovich[1], N. Kurt-Yilmaz[1], S Lee[2], R. Ishima[4], J. Luban[1], D. Bolon[1], C. Schiffer[1]

[1]UMass Medical School, Worcester, MA; [2]University of North Carolina School of Medicine, Chapel Hill, NC; [3]Schrodinger, Inc.; [4]School of Medicine, University of Pittsburgh

We hypothesized that mutations in the flaps and in regions outside the active site of the protease confer its drug resistance by altering the enzyme's flexibility and structure. However, mechanisms of the conformational changes upon mutation remain unknown presumably because only minor conformational changes are observed. To test the hypothesis, it is critical to establish the methodology to detect small conformational changes but yet may be sensitive to mutations or small differences in inhibitor pharmacophores. In combination of NMR and MD simulations, we approach to detect such small conformational changes that we could not be detected previously.

Systematic Exploration of Mutational Pathways to Drug Resistance in HIV Protease

R. Swanstrom[2], W. Sherman[3], A. Ali[1], K. Zeldovich[1], N. Kurt-Yilmaz[1], S Lee[2], R. Ishima[4], J. Luban[1], D. Bolon[1], C. Schiffer[1]

[1]UMass Medical School, Worcester, MA; [2]University of North Carolina School of Medicine, Chapel Hill, NC; [3]Schrodinger, Inc.; [4]School of Medicine, University of Pittsburgh

We investigated the interdependence of mutations involved in the evolution of drug resistance in HIV protease by designing a library with all possible combinations of an 11-fold strongly resistant mutant and analyzing how each mutant impacts protease function with and without drug selection. Our results reveal a rugged functional landscape where the effects of mutations are strongly dependent on the genetic background in which they occur.

Insights into HIV-1 Proviral Transcription from an Integrative Structure of the Tat:AFF4:P-TEFb:TAR Complex

Ursula Schulze-Gahmen[1], Ignacia Echeverria[2], Goran Stjepanovic[1], Yun Bai[1], Huasong Lu[1], Dina Schneidman-Duhovny [2], Jennifer A. Doudna[1,3,4,5], Qiang Zhou[1], Andrej Sali[2], and James H. Hurley[1,5]

[1]Department of Molecular and Cell Biology and California Institute of Quantitative Biosciences, University of California, Berkeley, Berkeley, United States; [2]Department of Bioengineering and Therapeutic Sciences, Department of Pharmaceutical Chemistry, and California Institute of Quantitative Biosciences, University of California San Francisco, San Francisco, United States; [3]Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, United States; [4]Department of Chemistry, University of California, Berkeley, Berkeley, United States; [5]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, United States

HIV-1 Tat hijacks the human superelongation complex (SEC) to promote proviral transcription. HIV-1 Tat forms a physical complex with P-TEFb (Cyclin T1 and CDK9), AFF1/4, and HIV-1 TAR RNA. Solution structures of peptide:TAR complexes and a crystal structure of Tat:AFF4:P-TEFb are known, but the structure of the active Tat:AFF4:P-TEFb:TAR complex is not. We used hydrogen-deuterium exchange, small angle x-ray scattering, and selective 2'-hydroxyl acylation analyzed by primer extension to determine the integrative structure of the complex. The structure reveals direct contacts between helix α2 of AFF4 and TAR, along with contributions of Cyc T1 TRM and Tat ARM to TAR major groove binding. Point mutations in helix α2 of AFF4 reduced TAR binding to Tat-AFF4-P-TEFb in solution, and the corresponding mutations in AFF1 reduce Tat transactivation in cell-based reporter assays. These findings provide a structural framework for a critical complex in the regulation of HIV-1 latency.

Arrayed Analysis of Immune Evasion: High Content Imaging Screen Reveals Novel Targets of HIV-1 Vpu

Guney Boso, Prashant Jain, Paul de Jesus, Quy Nguyen and Sumit Chanda

Sanford Burnham Prebys Medical Discovery Institute

One of the mechanisms by which HIV-1 evades immune restriction is through targeting of interferon stimulated genes (ISGs) such as APOBEC3G and BST-2 via viral accessory proteins. In an effort to test the hypothesis that there are additional ISGs that are targeted by HIV-1, we developed a high content imaging platform that enables the arrayed analysis of the stability of proteins in the presence or absence of a second protein. We assessed the performance of this platform to identify novel cellular targets of HIV-1 Vpu. We co-transfected 950 V5-tagged ISGs into HEK293 cells together with FLAG-tagged Vpu, or LacZ. Cells were then analyzed for V5 and FLAG staining intensities to reveal 40 proteins that were significantly destabilized after the expression of Vpu, but not LacZ. We confirmed degradation of a subset of these hits in the same system by immunoblotting analysis +/- proteasome inhibitor. We also found that at least five of these proteins are degraded in the HIV-1 infected cells in a Vpu dependent manner. One of these hits, UBE2L6 is an E2 ligase for ISG15 conjugation. Interestingly, we observed a Vpu dependent decrease in the levels of ISG15 conjugates in multiple cell types suggesting that HIV-1 targets this antiviral mechanism as an immune evasion strategy. Moreover we showed that overexpression of two of the hits (CD99 and PLP2) lead to a substantial decrease in HIV-1 infectivity. Our studies suggest that CD99 and PLP2 restrict viral infectivity by causing production of viral particles that are defective in cellular binding/entry.

Real-Time Imaging of Single HIV-1 Uncoating in Cells

Ashwanth C. Francis[1], Mariana Marin[1], Jiong Shi[2], Christopher Aiken[2] and Gregory B. Melikyan[1,3]

[1]Department of Pediatric, Emory University School of Medicine, Atlanta, GA; [2]Department of Pathology, Microbiology and Immunology, Vanderbilt University, Nashville, TN; [3]Children's Healthcare of Atlanta, Atlanta, GA

HIV-1 uncoating, a necessary step in infection, which involves shedding of the capsid protein from the core complex encasing the viral genome, is poorly understood. To elucidate this critical step of HVI-1 entry, we developed a novel strategy to visualize HIV-1 uncoating using a fluorescently tagged oligomeric form of a capsid-binding host protein cyclophilin A (CypA-DsRed), which is specifically packaged into virions through the high-avidity binding to capsid (CA). Single virus imaging reveals that CypA-DsRed remains associated with cores after permeabilization/removal of the viral membrane. Importantly, CypA-DsRed and CA are lost concomitantly from the cores *in vitro* and in living cells. The rate of CypA-DsRed loss is modulated by the core stability and is accelerated upon the initiation of reverse transcription. The majority of single cores lose CypA-DsRed shortly after viral fusion, while a small fraction remains intact for several hours. Single particle tracking at late times post-infection reveals a gradual loss of CypA-DsRed which is dependent on reverse transcription. Uncoating occurs both in the cytoplasm and at the nuclear membrane. To conclude, the novel CypA-DsRed based imaging assay enables time-resolved visualization of single HIV-1 uncoating in living cells and provides important clues regarding its spatio-temporal regulation.

More is Less: Enhancing Virus-Induced Membrane Fusion to Inhibit Viral Spread

Menelaos Symeonides[1,2], Evan T. Hoffman[1], Leslie A. Sepaniac[1], Alon Herschhorn[3], Joseph G Sodroski[3], Thorsten R. Mempel[4], and Markus Thali[1,2]

[1]Graduate Program in Cell and Molecular Biology, University of Vermont, Burlington, VT 05405, USA; [2]Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405, USA; [3]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02114, Massachusetts, USA; [4]Center for Immunology and Inflammatory Diseases, Division of Rheumatology, Allergy and Immunology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA

Interactions between the HIV-1 envelope glycoprotein (Env) on viral particles and the viral receptor/coreceptor on target cells trigger fusion of viral and host cell membranes, thus mediating virus entry. Transmission of viral particles occurs very efficiently if infected (virus producing) and uninfected (target) T lymphocytes transiently align, thus forming the so-called virological synapse (VS). When producer and target cell contact each other during VS formation they could, in principle, fuse and form a syncytium. Several safeguards put in place in virus producing cells, however, ensure that the majority of encounters between infected and uninfected cells resolve without fusion. Consequently, even if syncytia do form early in the infection process (as recently shown by intravital imaging of HIV-infected BLT mice by the Mempel group; *Nature* 2012; 490;283-7), they do not grow beyond the four or five nuclei stage. Based on this finding and also based on our more recent *in vitro* analyses of how fusion-regulating factors affect HIV-1 transmission, we hypothesize that enhancing Env-induced membrane fusion and thus promoting the formation of large T cell syncytia will impair viral spread.

Using fusion enhancers that were identified when the Sodroski lab performed a screen to isolate fusion inhibitors (*Nat Chem Biol* 2014; 10:845-852), we have started to test this idea by quantifying syncytia formation first in traditional 2D cell culture systems but then also in a simple 3D cell culture system of which we recently showed that it allows us to recapitulate HIV-1 Env-induced small syncytia *in vitro* (*Viruses* 2015; 7:6590-6603). Preliminary results of these studies, together with recent work by investigators studying varicella zoster virus (*Plos Path* 2014; 10:e1004173) or measles virus (*Genes Dev* 2015; 27:2356-2366), lend support to the concept that boosting (rather than repressing) membrane fusion, one of the key viral functions, can compromise virus production and thus that fusion enhancement merits to be considered as a potential anti-viral strategy.

Supported by 9R01GM117839-06 to MT

T1. Identifying and Incorporating Water-Mediated Interactions in Drug Discovery

Jiaye Guo,[1] and Robert C. Rizzo[2,3,4]

[1]Graduate Program in Biochemistry and Structural Biology, Stony Brook University, Stony Brook, New York 11794; [2]Department of Applied Mathematics & Statistics, Stony Brook University, Stony Brook, New York 11794; [3]Institute of Chemical Biology & Drug Discovery, Stony Brook University, Stony Brook, New York 11794; [4]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, New York 11794

In many disease-related systems, water-mediated interactions in the binding pocket play a critical role in fortifying hydrogen bonding between protein receptors and ligands. Polar functionalities in rationally designed ligands (*e.g.* cyclic urea inhibitors of HIV-1 protease) can mimic water-mediated interactions to improve binding affinity. The interaction energy contribution of bridging water molecules can be observed by comparing molecular footprints (defined as per-residue energy decompositions) computed with and without waters present. In this work we present a semi-automated protocol which aims to quantitatively identify important crystallographic water molecules ("bridging waters") that mediate hydrogen bonding between ligands and their protein targets. Knowledge from a set of reported bridging waters has been applied to refine the protocol. The protocol was subsequently applied to a test set of unexplored crystallographic water molecules to help identify potential bridging waters. The goal is to guide large-scale virtual screenings with molecular footprints of solvated ligands to identify new drug leads which mimic water-mediated interactions.

T2. A Genetic Algorithm for DOCK to Aid in *De Novo* Design

Courtney D. Singleton[1], Lauren E. Prentis[2], and Robert C. Rizzo[3,4,5]

[1]Department of Pharmacology, [2]Department of Biochemistry & Structural Biology, [3]Department of Applied Mathematics & Statistics, [4]Institute of Chemical Biology & Drug Discovery, [5]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

Genetic algorithms (GAs) are evolution-based optimization algorithms that have emerged as a powerful tool for atomic-level *de novo* design. Specifically, GA-based methods can be employed to construct novel small molecules with compatibility for biologically-relevant protein targets by chemically alter a starting ensemble through mating and mutations. The GA currently in development for the program DOCK6 incorporates 3D ligand geometry and binding interactions, as quantified by different scoring functions, to tailor the evolution of new molecules for improved binding in the context of a defined binding site. As proof-of-principle, the GA has been applied to several systems, including HIV glycoprotein 41 and HIV Protease, to evaluate the behavior of the method with various starting ensembles and binding site shapes. Progress in GA development and behavior on various protein-ligand systems will be presented.

T3. FightAIDS@Home Phase II: Refinement of Massive HIV Virtual Screening Experiments Using Large-Scale Molecular Dynamics Simulations

William F. Flynn[1,2], Junchao Xia[2], Nanjie Deng[2], Stefano Forli[3], Arthur Olson[3], and Ronald M. Levy[2]

[1]Department of Physics and Astronomy, Rutgers University, Piscataway, NJ; [2]Center for Biophysics and Computational Biology, Department of Chemistry, Temple University, Philadelphia, PA; [3]Molecular Graphics Laboratory, Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA

Using the volunteered computing power of IBM's World Community Grid (WCG), the TSRI-based FightAIDS@Home research team has conducted the world's largest virtual screening experiment over the past decade. By screening more than 20 billion compounds against HIV enzymes, the Olson laboratory has identified many potential hit molecules. These potential hits are now being refined using statistical mechanics-based, computationally intensive binding free energy calculations developed by the Levy group under FightAIDS@Home, Phase II. With nearly 100 volunteered CPU-years per day, Phase II of FightAIDS@Home aims to develop novel methodologies to improve enrichment in virtual screening and predict binding modes more accurately. FightAIDS@Home Phase II launched in October 2015, and the first computational experiments are testing different molecular dynamics (MD) sampling schema designed for the WCG's heterogeneous computing environment. Preliminary results for HIV-1 Integrase complexed with several ligands show binding free energies computed on the WCG are qualitatively consistent with replica exchange molecular dynamics (REMD) simulations on conventional HPC hardware, and the massive scale of the computations allow simulations to overcome free energy barriers in conformational space that may restrict conventional simulations from exploring correct binding modes.

This work would not be possible without the support of IBM. Please visit https://secure.worldcommunitygrid.org/about_us/viewAboutUs.do for more information.

T4. Animating the Science of HIV

Janet Iwasa

Department of Biochemistry, University of Utah

The Science of HIV project seeks to create scientifically accurate and visually stunning animations of the HIV life cycle, highlighting the findings of the P50 Centers for HIV/AIDS-Related Structural Biology. The primary method of outreach to the public will be a multimedia-rich website (http://ScienceofHIV.org). The final animation, which will be approximately 5-10 minutes in length, will tell the compelling story of how a virus is able to hijack its host cell in molecular detail. The visualization will be made available to students, educators and the public in a variety of media contexts, and may act as a powerful means to display the impressive progress that has been made in understanding the molecular mechanisms of HIV infection.

T5. Coarse-grained (CG) Computer Models of Key Stages in the HIV-1 Lifecycle

J. M. A. Grime and G. A. Voth

[1]*Department of Chemistry, Institute for Biophysical Dynamics, James Franck Institute, and Computation Institute, The University of Chicago, Chicago, Illinois 60637, USA*

The HIV-1 viral lifecycle requires the generation and release of a viral particle, or virion, from an infected cell. Prior to viral particle release, several thousand copies of the Gag polypeptide aggregate to form an interconnected lattice at the cytosolic leaflet of the infected cell membrane. This “immature” lattice helps to package viral RNA, and assists in the budding of an immature viral particle. Subsequent to viral particle release, a complicated process of maturation converts the initially non-infectious viral particle into the mature and infectious form. Maturation includes the self-assembly of many copies of the capsid protein to generate a “capsid” structure that surrounds the viral RNA. The lack of a suitable capsid precludes viral infectivity.

In order to study the critical stages of the HIV-1 lifecycle, we use simplified “ultra-coarse-grained” (UCG) computational models of key molecular components to examine various self-assembly behaviors. The simulations recapitulate various supramolecular structural elements, suggesting that the application of such simplified models can provide a valuable complement to experimental studies of viral maturation.

T6. Modeling Affinity Maturation of Anti-HIV Antibodies Targeting gp120

Dzmitry Padhorny and Dima Kozakov

Laufer Center for Physics and Structural Biology, Stony Brook University

Understanding antibody affinity maturation on structural level is of great importance for immunology, particularly for the design of efficacious humoral vaccines for influenza and HIV-1. In this work we predict the changes in binding affinity due to amino acid mutations during affinity maturation. This is done by calculation of approximate interaction partition function of antigen-antibody interactions using Fast Fourier Transform based sampling with accurate molecular mechanics based potentials. We present our results on the data on neutralization of Zm176.66, Ker2018.11, Q23.17, Yu2 and Q842.d12 HIV strains by a series of broadly neutralizing anti-HIV antibody variants targeting gp120 protein. Our prediction results show good correlation with experiment, and while further development and testing is required, this demonstrates that *in silico* structural modeling of antibody maturation is feasible.

T7. The Broadest bnAbs Target More Conserved HIV-1 Env Epitopes

Hongjun Bai, Merlin L. Robb, Nelson L. Michael and Morgane Rolland

US Military HIV Research Program, Silver Spring, Maryland 20910, USA

HIV-1 vaccines that could trigger the maturation of broadly neutralizing antibody responses (bnAbs) would likely be potent. To gain insight for vaccine design, we analyzed the relationship between HIV-1 Env diversity and the neutralization breadth of bnAbs.

We characterized HIV diversity in datasets of independent sequences corresponding to HIV-1 subtypes A1 (n = 229), B (n = 1,302), C (n = 972) and CRF01_AE (n= 454) and a group M dataset reflecting the global distribution of HIV-1 sequences (n = 241). We estimated the Ab accessibility of each Env site based on the Env trimer 4TVP and found that sites accessible to Ab (n = 324) were more variable than other Env sites (Shannon Entropy = 0.41 vs 0.05, $p < 0.001$).

Next, we focused on the contact sites on Env that defined the epitopes corresponding to 17 bnAbs and analyzed the relationship between the epitope diversity and the neutralization breadth of the bnAbs (as determined experimentally (Georgiev, Science, 2013). There was no relationship between epitope diversity and neutralization breadth of bnAbs (Spearman $\rho = -0.022$, $p = 0.930$). However, when we took into account the number of Ab atoms contacting each site of the epitope, we found that the broadest bnAbs targeted less diverse epitopes ($\rho = -0.683$, $p = 0.003$ for gp M dataset; subtype specific results were similar: 0.497 (HIV-1 C) $\leq \rho \leq 0.711$ (HIV-1 A1), $p \leq 0.044$).

These results highlight how bnAbs target conserved epitopes with strong interactions between the bnAbs and the contact residues on Env. While at the individual level, HIV-1 diversification is critical to the development of bnAbs, we found that, at the population level, the broadest bnAbs target the most conserved epitopes, thereby potentially allowing cross-reactivity with heterologous Env proteins. These findings support vaccine strategies focusing on conserved elements of the virus.

T8. Structural Analysis of an HIV-1 Broadly Neutralizing V3-Glycan B-cell Lineage

Fera D[1], Bonsigniori M[2], Kreider E[3],Meyerhoff R[2], Bradley T[2], Wiehe K[2], Alam SM[2], Hwang KK[2], Saunders KO[2], Zhang R[2], Gladden MA[2], Monroe A[2], Kumar A[2], Xia SM[2], Cooper M[2], Jette CA[1], Pier BW[1], Montefiori DC, Trama A, Liao HX[2], Kepler TB[4], Gao F[2], Shaw GM[3], Hahn B[3], Moody MA[2], Gao F[2], Mascola JR, Haynes BF[2], and Harrison SC[1,5]

[1]Laboratory of Molecular Medicine and [5]HHMI, Boston Children's Hospital, Harvard Medical School, Boston, MA; [2]Department of Medicine, Duke University School of Medicine, Duke University Medical Center, and Duke Human Vaccine Institute, Durham, NC; [3]Departments of Medicine and Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [4]Departments of Microbiology, and Mathematics and Statistics, Boston University, Boston, MA; [5]Vaccine Research Center, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Proposed strategies to elicit HIV broadly neutralizing antibodies (bnAbs) require a deeper understanding of the evolution of the immune response to vaccination or infection. In HIV infected individuals, viruses and B-cells evolve together, creating a virus-antibody "arms race." We describe here an analysis of the virus-antibody arms race for a V3-base, glycan-dependent bnAb lineage, called DH270. DH270.1 is a bnAb that depends, like other bnAbs of its class, on the N332 glycan for binding and neutralization. N332 dependent bnAbs also rely on different envelope (*Env*) segments for their interaction, and so they have different binding footprints. To understand antibody affinity maturation in the DH270 lineage, we determined crystal structures of Fabs for the UCA, several mature members, and a mature member in complex with a man-9 glycan. A low resolution negative stain electron microscopy (EM) 3D reconstruction was also determined for DH270.1 in complex with HIV *Env*.

Structural analysis of DH270.1 and other lineage members revealed a similar overall architecture, and suggests that development of broad neutralization of this lineage may have arisen due to changes in the biochemical nature resulting from amino acid side chain changes. Binding kinetics of mutants of DH270.1 and *Env*, together with a crystal structure of man-9 glycan in complex with a more mature form of DH270.1, and a negative stain EM 3D reconstruction of DH270.1 with a SOSIP.664 *Env* trimer, indicate that three DH270.1 Fabs bind to one trimer with a distinct binding footprint when compared to other V3-glycan bnAbs. Higher resolution structures using cryo-electron microscopy are currently underway to provide greater insights into the evolutionary arms race between the DH270 lineage, cooperating antibody lineages (from the same infected individual) that aided the development of the DH270 lineage, and the infecting virus. These data will further suggest how to design immunogens to induce bnAb development.

Supported by NIH/NIAID-funded Center For HIV/AIDS Vaccine Immunology-Immunogen Discovery grant (CHAVI-ID; UM1 AI100645) and F32 fellowship (1F32AI116355-01).

T9. A Protective Role for CD169 in Limiting Systemic Spread of a Pathogenic Retrovirus

Pradeep D. Uchil[1], Ruoxi Pi[1], John D. Ventura[1], Kelsey A. Haugh[1], Xaver Sewald[2], and Walther Mothes[1*]

*[1]Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06510; [2]Max von Pettenkofer-Institute, Ludwig-Maximilians-University Munich, Munich, Germany; *corresponding author*

CD169 expressed on surfaces of subcapsular sinus and metallophillic macrophages can capture and promote retrovirus trans-infection of B cells. Whether this process is protective or detrimental to the host is not known. Here, we used pathogenic splenomegaly-inducing retrovirus, the Friend Virus Complex (FVC) to study how CD169 shapes the outcome of retrovirus infection. Our data revealed that while CD169 promoted infection at the primary draining lymph node, surprisingly, it limited systemic retroviral spread. Interfering with CD169-function enhanced viral dissemination to secondary draining sites and accelerated mortality in susceptible mouse strains. This was due to predominant infection of erythroblasts where FVC manifests its pathogenesis in absence of CD169-directed delivery of viruses to lymphocytes. In contrast, both efficient infection and spread of non-pathogenic Friend MLV was primarily CD169-dependent as it targets lymphocytes and not erythroblasts for replication. Thus, our data reveal that CD169 plays a protective role in the host because it limits systemic spread and restricts virus infection locally to primary draining lymph nodes.

T10. Functional Interplay Between Murine Leukemia Virus Glycogag, Surface Glycoprotein and Serinc5 Modulates Virus Entry

Yadvinder S Ahi[1], Shu Zhang[1], Delphine Muriaux[2], Amin Feizpour[3], Björn M Reinhard[3], Rahm Gummuluru[3], and Alan Rein[1]

[1]NCI, HIV DRP, Frederick, MD; [2]CNRS, Membrane Domains and Viral Assembly, Montpellier, France; [3]Boston University, Boston, MA

Murine leukemia virus (MLV) is a prototypical gammaretrovirus. In addition to the three polyproteins, Gag, Pol and Env, many gammaretroviruses also code for “Glycogag” (gGag), translated from a CUG codon located upstream and in-frame with the Gag open reading frame. gGag significantly enhances MLV infectivity, but this effect depends on the type of Env glycoprotein on the virus (Pizzato, 2010). Remarkably, gGag complements Nef deficient HIV-1 by protecting the virus from the novel host restriction factors Serinc5 and Serinc3 (Rosa et al, 2015; Usami et al, 2015).

We showed that gGag traffics through endoplasmic reticulum and Golgi. We also confirmed the finding of Pizzato that gGag significantly enhances the infectivity of virus with xenotropic and amphotropic, but not ecotropic Env. We found that gGag enhances the infectivity of the virus with 10A1, GALV and RD114 Envs. In contrast, however, gGag was found to be detrimental to the infectivity of virus with Ebola glycoprotein. The infectivity of MLV(xeno) lacking gGag could be rescued in trans by a remarkably low amount of gGag expression plasmid, as if gGag acts in a catalytic fashion to enhance infectivity. We also found that the S2 protein of equine infectious anemia virus could replace gGag in these experiments.

We developed an extremely sensitive assay to measure entry of MLVs. Using this assay, we found that gGag affects the virus infectivity by impacting its entry into target cells. We found that Serinc5 blocks infectivity of MLV(Xeno) lacking gGag. On the other hand, Serinc5 improves the infectivity of MLV(Ebola) with gGag. Thus, the counteraction of serinc5 by gGag results in contrasting effects on the infectivity of the virus with xenotropic and Ebola glycoprotein. It is possible that gGag alters the lipid composition of the virus particles, thus impacting virus entry mediated by only certain Envs. Understanding how the lipid profile of MLVs is altered by gGag and Serinc5 may provide insight into the mechanism of gGag function.

T11. Multi-Dimensional Profiling of Primary Human CD4+ T Memory Cells

Lara Manganaro[1], Jeffrey R. Johnson[2,3,4], Patrick Hong[1], Benhur Lee[1], Nevan Krogan[2,3,4] and Viviana Simon[1,5,6]

[1]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [2]Gladstone Institute of Virology and Immunology, 1650 Owens Street, San Francisco, CA 94158, USA; [3]University of California, San Francisco, CA 94158, USA; [4]QB3, California Institute for Quantitative Biosciences, San Francisco, CA 94158, USA; [5]Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA; [6]Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, New York, USA

Human CD4+ T cells constitute the long-lived HIV reservoir, which prevents virus eradication in HIV infected patients treated with highly active antiretroviral therapy (HAART). Emerging evidence suggests that the HIV reservoir is formed early upon infection within the CD4+ T memory cell population. The CD4+ T memory cell population is heterogeneous with some cells maintaining the ability for self-renewal (central memory or stem cells). We hypothesized that the different CD4+ T memory cells differ with respect to their ability to support productive infection, cell cycle and proliferation status.

We used Cytometry by Time of Flight (CyTOF) to compare the immuno-phenotype, cell cycle profiles and proliferation capacity of primary human CD4+ T cells stimulated with IL2 or IL15 and infected with replication competent HIV. A custom antibody cocktail was used to detect >20 surface and intracellular markers including HIV p24 using sample multiplexing. In addition, we performed quantitative phospho-proteomics of primary human CD4+ T cells treated with IL2 and IL15 in order to define signaling pathways specific for the given immuno-phenotype.

The CyTOF analysis revealed that T memory stem cells (T_{SCM}) display high proliferation capacity, which was further amplified in the presence of IL15. Moreover, T_{SCM} efficiently supported HIV infection. The susceptibility to infection was enhanced by IL15 with T_{SCM} constituting up to 9% of the infected cell population. Cell cycle analysis showed a skewing of the infected cell populations towards the G_2 phase.

The phosphoproteomic analysis of CD4+ T cells found that 231 proteins were differentially phosphorylated in IL15 compared to IL2. The main processes activated by IL15 were RNA metabolism, transcription regulation, chromatin remodeling, signal transduction and cytoskeleton remodeling. Of note, we also detected the up-regulation of SAMHD1 phosphorylation upon IL15 stimulation.

Our data indicate that T_{SCM} are cycling and support productive infection. Moreover, the T_{SCM} pool is dynamic and expands in response to IL15. This cytokine activates several pathways favorable to infection (transcription and chromatin remodeling) and counteracts the antiviral activity of SAMHD1. A global characterization of the features of infected T memory cells including CD4+ T stem cells is a key factor for the development of new strategies to eliminating the reservoir.

T12. Understanding Glycan Type Specificity in Highly Glycosylated Proteins

Artem Krantsevich and David F. Green

Stony Brook University

Despite the fact that the vast majority of glycans in most known glycoproteins are of complex type, some glycoproteins have a clear tendency to contain high-mannose and hybrid structures as well. For example, HIV envelope glycoprotein gp120 usually has about 24 glycans, and almost half of them are either high-mannose or hybrid. Statistical analysis of gp120 suggests that: 1) glycosylation sites placed closer to the C-terminus of the protein tend to carry high-mannose/hybrid glycans; 2) glycosylation sites placed in flexible loops tend to carry complex glycans; and 3) glycosylation sites placed in regions of secondary structure tend to carry high-mannose/hybrid glycans. In order to develop a mechanistic understanding of the origin of these differences, we developed a stochastic chemical-kinetic model of the N-linked glycosylation pathway in the Golgi Apparatus. This model provides some understanding of how the distribution of glycan types depends on: 1) concentration of glycans in the Golgi Apparatus; 2) input to the Golgi Apparatus; and 3) parameters that describe certain glycan modifications.

T13. Insights into Structures and Dynamics of Variable Regions of Major Subtypes of HIV-1 Gp120

Tuoling Qiu[1], and David F. Green[1, 2]

[1]Chemistry Department, Stony Brook University, Stony Brook, NY 11794; [2]Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY 11794

HIV-1 envelope glycoprotein gp120 subunits, together with transmembrane glycoprotein gp41, form trimeric spikes on the viral surface. These spikes play a crucial role in initiating HIV-1 infection by binding of gp120 to CD4 receptor and subsequently to co-receptors (CCR5 or CXCR4) on target cells. Gp120 consists of 5 functionally important hypervariable regions (V1–V5) that are highly flexible and heavily glycosylated. However, all the previously reported crystal structures of gp120 are missing part of the variable regions, especially for V1 and V4. Here, we constructed gp120 models with complete variable regions for major subtypes of HIV-1. The models were produced with a combination of ab initio modeling and knowledge based methods using MODELLER program. Models were selected and verified with DOPE potential, PROCHECK and other tools. Our models in general show well folded V2-V3 beta barrel structures and relatively disordered V1 and V4 regions. Models were then carefully minimized and subjected to molecular dynamics (MD) simulations in order to investigate the conformational mobility of variable regions, especially the V2-V3 beta barrel structures, with or without the binding of antibodies.

T14. Mapping the Conformational Space of Glycoconjugate-Linked Carbohydrates

Xindi Li[1] and David F. Green[1, 2, 3, 4]

[1]Department of Applied Mathematics & Statistics, [2]Department of Chemistry, [3]Laufer Center for Physical and Quantitative Biology, [4]Graduate Program in Biochemistry and Structural Biology, Stony Brook University, Stony Brook, New York 11794-3600, United States

It is well understood that carbohydrates and glycoconjugates play crucial roles in biological processes such as coagulation, fertilization, apoptosis and immunity, yet they still remain understudied. Saccharides' conformational flexibility, especially considering their nonlinear backbones, anomeric carbons, and different glycosidic linkage points with various monosaccharide units, increase the difficulty of fully understanding their behaviors in glycan-involved interactions. However, their functional specificity, such as recognition between glycans and other glycans, enzymes or lectins, is highly dependent on their conformational preferences. Hence the accurate prediction of glycan conformations is becoming more and more urgent in carbohydrate chemistry.

In particular, HIV glycoprotein gp120 is densely covered with glycans which account for half of its total mass and several families of broadly neutralizing antibodies (bnAbs) has been discovered showing inhibition for HIV infection in cell culture through binding to different glycan moieties. Unfortunately, available experimental data is far from enough and interpretations about them have no uniform criteria and bear intrinsic drawbacks. However, using molecular mechanism methods with libraries of peptides rotamers, we are able to generate enough meaningful data and gain insights into structures and functions of proteins and nucleic acids. Hence, to set up libraries for glycans, our approach is to get detailed maps of the free energy landscape for all the biologically important disaccharides and trisaccharides and then explore them using statistical models such as Gaussian Mixture model (GM) and bivariate Von Mises Mixture (VM) model. Instead of molecular dynamics (MD) simulations which would only visit certain conformations during a typical MD run, we use Monte Carlo (MC) simulation to sample from the whole conformational space. Our preliminary results from oligosaccharide simulations show clear convergence patterns for glycosidic linkages. Both GM model and VM model work well in describing their distributional characteristics while the latter also provides estimates that properly account for the circular nature of angular data.

T15. Deciphering the Mechanisms of HIV-1 Entry Using Novel Env Heterotrimers

Mukta D. Khasnis and Michael Root

Department of Biochemistry and Molecular Biology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA

The process of HIV-1 entry involves fusion of the viral and cellular membranes, and is mediated by the viral surface glycoprotein Env (gp120/gp41), which is arranged as a trimer-of-heterodimers. The gp120 subunits interact with CD4 and chemokine receptors (CoR) and coordinate structural changes in the gp41 subunits, which in turn catalyze membrane fusion. Each Env trimer contains three CD4 and three CoR binding sites, but entry requires less than three binding events for each of these cellular proteins. Here, we developed a strategy to assess the impact of multiple CD4 and CoR binding events on Env trimer activity. HIV-1 containing Env heterotrimers were generated from virus-producing cells that expressed two Env species: one of which is defective in binding to CD4 or CoR, and the other which contains mutations in the cytoplasmic tail domain (CTD) of gp41 that impair Env incorporation into budding virions. We generated two novel Env heterotrimers which allowed us to study the impact of either 1) a single CD4 binding event in the context of multiple possible CoR binding events, or 2) a single CoR binding event in the context of multiple possible CD4 binding events. As expected, inhibitor titrations showed the amount of CD4 binding per trimer influenced CoR binding. Surprisingly, we also found that the amount of CoR binding per trimer impacts CD4 binding. We were also able to alter the levels of CD4 and CoR on target cells through the use of the binding antagonists D23.2 and AMD3100, respectively. We are currently generating infectivity profiles by applying HIV-1, which contain our novel Env heterotrimers, to these target cells. These infectivity profiles will inform us of subtle changes in receptor-mediated HIV-1 entry when Env can only perform minimal CD4 or CoR binding events.

T16. Structural Dynamics of HIV Env Glycoproteins: A Link Between Structural, Functional, and Phenotypic Variation Among Isolates

Kelly K. Lee[1], Hans Verkerke[1], Miklos Guttman[1], James A. Williams[1], Yu Liang[1], Thaddeus M. Davenport[1], Cassie Simonich[1,2], Shiu-Lok Hu[1], and Julie Overbaugh[1,2]

[1]University of Washington, [2]Fred Hutchinson Cancer Research Center, Seattle, WA

The HIV-1 envelope glycoprotein (Env) mediates viral entry into host cells and is the sole target of neutralizing antibodies. While functional differences such as receptor reactivity and antigenicity are prevalent among Env from diverse HIV-1 isolates, we have only a nascent understanding of the structural basis for those differences. This gap in understanding stems from the limited number of native-like trimeric forms of Env that are available for structural analysis. In addition, the constructs that have been studied were in part selected for their relative stability and compatibility with high-resolution structure determination methods such as X-ray crystallography and cryo-EM. The recent near-atomic resolution structures of the engineered native-like Env trimers ("SOSIP" trimers) have provided unprecedented insight into Env architecture, revealing how the subunits interact, delineating neutralization epitopes, and even resolving significant portions of the glycan shield. The static structures, however, only tell a part of the story. Under native conditions, the Env fusion glycoprotein is animated by structural dynamics at all levels of its organizational hierarchy. Even at equilibrium, the trimeric complex samples a range of conformational states, transiently exposing or disrupting conformational epitopes. It is in these more dynamic aspects that largely define key elements of variation among Envs and hence viral phenotypes.

To understand the structural basis for HIV Env diversity, one needs to examine a wide range of Env trimers from divergent isolates and structural analytical tools that are amenable to studying the glycoprotein complexes under native solution conditions where differences are more likely to be evident. Using a novel purification approach for isolating native-like soluble trimers based upon their biochemical properties (exposure of charged and hydrophobic surfaces as well as size) rather than specific antigenic traits (as current mAb-based purification approaches require), we have been composed a panel of native-like SOSIP-engineered Env trimers from diverse HIV-1 isolates sampled from clades A, B, and C. To study the Env assemblies under native conditions, we use a set of analytical techniques including Hydrogen/Deuterium-exchange mass spectrometry (HDX-MS), X-ray generated radical footprinting, small-angle X-ray scattering, and electron microscopy. In a series of studies using HDX-MS and quantitative antibody-Env binding assays, we have resolved significant isolate-specific differences in local epitope order among Envs, and we demonstrated that local structural dynamics of conformational epitopes inversely correlates with the association rate of antibodies targeting those epitopes. Isolate-specific differences in Env dynamics thus directly impact its antigenic profile.

Through these integrative analytical approaches, we are beginning to identify the links between structure, dynamics, isolate-specific variation, and antigenicity. We see this information as providing a structure-based framework for understanding viral phenotypes. It also will aid in the optimization of Env-based vaccines.

T17. On the Role of the V3 Loop in the Conformational Thermodynamics of Bridging Sheet Formation in HIV-1 Gp120: On-the-fly Parameterization Free-energy Calculations of the SOSIP BG505 Protomer

Alexis Paz[1], Matthew Cameron[1], and Cameron F. Abrams[1,2]

[1]Department of Chemical and Biological Engineering, Drexel University, 3141 Chestnut St., 19104, Philadelphia, PA (US); [2]Department of Biochemistry and Molecular Biology, Drexel College of Medicine, 25 North 15th Street, Philadelphia, PA (US)

An important structural feature of the CD4-bound form of the HIV-1 envelope glycoprotein gp120 subunit is the so-called "bridging sheet", which is an ordered array of the β2, β1, β21, and β20 strands collectively acting as the requisite co-receptor binding site. In the recent crystal structures of the soluble trimeric SOSIP BG505 constructs [1], the bridging sheet is not formed, with the structure suggesting that flipping of the β1 – β2 motif by 180 degrees around its long axis would lead to its formation. However, this might require substantial rearrangement of the V1/V2 loop that separates β1 and β2. To better understand the thermodynamic requirements of bridging sheet formation in the SOSIP context, we performed all-atom explicitly solvated molecular dynamics (MD) simulations and related free-energy calculations, using the method of on-the-fly free-energy parameterization (OTFP) [2]. For expediency, we consider a single gp120 protomer excluding gp41 residues. We indeed show that it is energetically prohibitive to form bridging sheet in SOSIP because it is resisted by the lack of motion of V1/V2 to accommodate the flip. However, a major contribution to this structural rigidity of V1/V2 is its interface with the V3 loop, and we reasoned that displacing the main body of the V3 loop away from the surface of the core protein would free the V1/V2 to be flexible enough to allow the β1 – β2 flip. After displacing the V3 loop using steered MD, we found that the state in which bridging sheet is folded is indeed the thermodynamically most stable conformation. The free energy change upon formation is similar to that of core monomer gp120 with no V1/V2 loop. Our results suggest that CD4 encounter with SOSIP gp120 is alone not sufficient to trigger bridging sheet formation, but that V3 must also first be deployed from its native conformation embedded in a cavity formed by V1/V2. This in turn raises the possibility that the envelope conformational cascade associated with entry begins first with V3 deployment and interaction with co-receptor *before* CD4 encounter. We speculate that the V1/V2-V3 interface therefore possibly represents an interesting new target for compounds that might allosterically inhibit viral entry.

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T18. Single-Molecule FRET Delineates Asymmetric Trimer Conformations During HIV-1 Entry

Xiaochu Ma[1], Alon Herschhorn[2,3], John D. Ventura[1], Daniel S. Terry[4], Jason Gorman[5], Jonathan R. Grover[1], Xinyu Hong[1], Zhou Zhou[4], Hong Zhao[4], Roger B. Altman[4], James Arthos[6], Peter D. Kwong[5], Joseph Sodroski[2,3,7], Scott C. Blanchard[4], Walther Mothes[1*], and James B. Munro[8*]

*[1]Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06536, USA; [2]Department of Cancer Immunology and Virology, Dana-Farber Cancer Institute, Boston, MA 02215, USA; [3]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA 02115, USA; [4]Department of Physiology and Biophysics, Weill Cornell Medical College of Cornell University, New York, NY 10021, USA; [5]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA; [6]Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA; [7]Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health, Boston, MA 02115, USA; [8]Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111, USA; *Correspondence to: Walther Mothes (walther.mothes@yale.edu) and James Munro (James.Munro@tufts.edu)*

HIV-1 entry into cells requires binding of the viral envelope glycoprotein (Env) to receptor CD4 and coreceptor. Imaging of individual Env molecules on native virions revealed that Env trimers are dynamic, spontaneously transitioning between three distinct conformations. Binding of CD4 and coreceptor surrogate antibody 17b promotes opening of the closed Env (State 1) to stabilize an activated conformation (State 3) by way of at least one structural intermediate (State 2). Here, using single-molecule Fluorescence Resonance Energy Transfer (smFRET), we identify this intermediate as an asymmetric conformation where only a single CD4 molecule engages the Env trimer and individual protomers adopt distinct conformations. We further show that complete activation is enhanced by oligomeric CD4, suggesting that local clustering of the receptor may promote entry.

T19. Investigating Conformational Transitions in HIV-1 Env Using Combinations of CD4 Antagonists, Chemokine Receptor Antagonists and Fusion Inhibitors

Koree W. Ahn and Michael J. Root

Thomas Jefferson University, United States of America

HIV-1 entry proceeds through large structural changes in the trimeric viral glycoprotein Env (gp120/gp41). The interaction of gp120 subunits with cellular CD4 and chemokine receptors coordinates gp41 conformational transitions that culminate in fusion of viral and cellular membranes. Entry can be blocked by antagonists of CD4-gp120 interaction (RAs, e.g. darpin D23.2), antagonists of chemokine receptor-gp120 interaction (coRAs, e.g. AMD3100, TAK779) and inhibitors of gp41 conformational changes (fusion inhibitors, e.g. C37, 5-Helix). Our previous work on the activity of coRAs/fusion inhibitor combinations confirmed that synergy arises from coRA-dependent alterations in fusion kinetics and depends on fusion inhibitor target site, fusion inhibitor affinity and the stoichiometry of chemokine receptor-Env engagement. Here, we investigated the activities of RA/fusion inhibitor and RA/coRA combinations. We hypothesized that both inhibitor combinations would display synergistic activity since CD4 binding exposes chemokine receptor binding sites on gp120, which, in turn, control fusion kinetics. Indeed, we found that D23.2 and C37 displayed a robust synergy against Env_{HXB2} that mirrored the properties of AMD3100/C37 synergy, including dependence on the stoichiometry of CXCR4 binding. However, contrary to our hypothesis, D23.2 and AMD3100 displayed antagonistic activity against Env_{HXB2}. Furthermore, the synergistic and antagoinistic activities of RA/fusion inhibitor and RA/coRA combinations were dependent on viral strain, unlike the synergistic activity previously observed for coRA/fusion inhibitor combinations. Our findings minimized the importance of CD4-induced exposure of chemokine receptor binding sites in determining the activity of RA drug combinations. Instead, combinatorial activities correlated better with CD4-induced gp120 shedding, suggesting that synergy/antagonism arises from kinetic competition between Env inactivation, gp120-chemokine receptor binding, and gp41 structural changes. Implications for the role of CD4 and chemokine receptor binding in coordinating Env structural changes are discussed.

Funded NIH R01 GM66682 to MJR

T20. Cyclic Peptide Triazole Rigid-Receptor Docking and Molecular Dynamics Simulation in Three Different Gp120 States: Comparison of Targets for Future HIV-1 Antagonist Optimization

Francesca Moraca[1], Adel A. Rashad[2], Kriti Acharya[2], Irwin Chaiken[2] and Cameron F. Abrams[1]

[1]Department of Chemical and Biological Engineering, Drexel University, 3141 Chestnut St., 19104, Philadelphia, PA (US); [2]Department of Biochemistry and Molecular Biology, Drexel College of Medicine, 25 North 15th Street, Philadelphia, PA (US)

AIDS is caused by the Human Immunodeficiency Virus type 1 (HIV-1) and is a serious health concern worldwide. HIV-1 cell entry is gated by surface glycoprotein envelope spike (Env) trimers of gp120/gp41 heterodimers. The entry mechanism begins with binding of cellular CD4 to gp120, which conformationally decrypts a binding site on gp120 for the co-receptor (CCR5 or CXCR4). Co-receptor binding triggers a conformational cascade that exposes gp41 N-terminal peptide for insertion into the target cell membrane, followed by a refolding event that brings the viral and cell membranes into close proximity to promote virus-cell fusion. Molecules designed to interfere with any one of these steps are collectively referred to as entry inhibitors. Among them, peptide triazoles (PT) are known to bind to gp120, inhibit binding at both CD4 and co-receptor sites on the Env, trigger gp120 shedding and therein lead to irreversible inactivation of the virus before cell encounter. Linear and metabolically stable cyclic peptide triazoles (PT and cPT, respectively) have been extensively studied in our group both theoretically and experimentally, using a combination of mutagenesis experiments, synthetic design, conformational analysis, docking and molecular dynamics (MD) simulations. Here we present a conformational analysis, rigid-receptor docking and MD simulation of the cPT AAR029F in 4 different available targets: (i) monomeric gp120/SMCM complex (PDB ID: 4I53 – target 1); (ii) trimeric SOSIP (PDB ID: 4NCO – target 2); (iii) monomeric gp120/F105 complex (PDB ID: 3HI1 – target 3); (iv) trimeric JR-FL (PDB ID: 5FUU – target 4). AAR029F is characterized by a prototypic PT pharmacophore I-X-W, where X is adamantyl triazolePro. We sought to identify suitable protein targets to guide further PT/cPT optimization given the current lack of a gp120-PT/cPT co-crystal structure. Target 1 was used as a negative control, since it is known that PTs bind before the formation of the bridging sheet (present only when gp120 is in the CD4-bound state, namely the activated state), and inhibit its formation. Docking in targets 2 and 3 gave structures in line with the putative mechanism of action of preventing bridging sheet formation and also consistent with the effects of mutagenesis on binding. The docking on target 4 has been performed in all the 3 protomers of the trimer, since they are not identical. Comparing the first docking pose in protomers A, B and C, only the docking in protomer C showed results in line with the previously performed mutagenesis experiment. The MD simulations of AAR029F/5FUU-protomer C and AAR029F/3HI complex are ongoing. The MD simulation on AAR029F/4NCO shows that the cPT induces a stabilization of the protein in its binding site relative to the *apo* protein. In conclusion, all docking complexes in targets 2 to 4 show that the I-X-W pharmacophore is responsible for major contacts of the cPT to the protein. To discriminate among targets 2, 3 and 4, future work will involve performing Free Energy Perturbation (FEP) simulations in all complexes, namely AAR029F/4NCO, AAR029F/3HI1 and AAR029F/5FUU-protomer C, upon mutating S375 and W112 and computing $\Delta\Delta G$'s of binding. Previously mutagenesis studies demonstrated that those two residues are important for PT binding to SOSIP, and we will estimate the impact of their mutation in the most stable AAR029F/4NCO, AAR029F/3HI1 and AAR029F/5FUU-protomer C complexes derived from the MD simulations.

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T21. Generation and Characterization of HIV-1 Escape Mutants to Peptide Triazole Entry Inhibitors

Andrew P. Holmes[1,2], Adel Ahmed[2], Lauren D. Bailey[2], Katie Kercher[1], William Dampier[1], Michael Nonnemacher[1], Michael Root[3], and Irwin Chaiken[2]

[1]Department of Microbiology and Immunology, Drexel University College of Medicine; [2]Department of Biochemistry and Molecular Biology, Drexel University College of Medicine; [3]Department of Biochemistry and Molecular Biology, Thomas Jefferson University

We have initiated a study to evolve resistant viruses to multiple classes of peptide triazole entry inhibitors in order to understand the mechanisms of escape and inactivation. The macrocyclic and sulfhydryl-containing peptides used in this study are both capable of inactivating the virus through gp120 shedding, but the latter class has the additional ability to induce cell-free virolysis. Generation of HIV-1 escape mutants was accomplished through virus passaging on an immortalized T-cell line with dose escalation of inhibitor. Over the course of approximately four months, we were able to establish resistant virus cultures that grow under peptide concentrations that are two orders of magnitude greater than the starting concentration. Traditional Sanger sequencing of the escape mutant HIV-1 genome revealed a similar escape profile for each class despite the difference between their inactivating effects. Additionally, deep sequencing was carried out to quantitatively monitor the evolution of various quasispecies in each culture and determine the likelihood that mutations arise together. Mutations within the binding site and around the HIV-1 Env protein were discovered, including at the gp120-gp41 interface and the trimer apex, which supports our hypothesis that peptide triazoles actuate inactivating pressure throughout the protein complex. From this pool of mutations, we generated individual and combinational mutations in a pseudoviral system by site-directed mutagenesis to investigate the mechanism of escape. We have shown that small changes in the side chain at a pivotal location between the peptide binding cavities, V255I/T, have a profound effect on inhibitor function. We believe these mutations restrict conformational perturbation necessary for inactivation, which is likely caused by affecting inhibitor binding as evidenced by molecular docking on the trimer in the presence and absence of the V255 mutations.

T22. Optimization of Macrocyclic Peptide Triazole HIV-1 Inactivators

Adel Ahmed Rashad*, Kriti Acharya*, Rachna Aneja, Ann Haftl and Irwin Chaiken

*Department of Biochemistry & Molecular Biology, Drexel University College of Medicine, Philadelphia, Pennsylvania 19102; *Corresponding authors: adel.ahmed@drexelmed.edu, ka536@drexel.edu*

HIV-1 entry inhibition remains an urgent need for AIDS drug discovery and development. We previously reported the discovery of cyclic peptide triazoles (cPTs) that retain the HIV-1 irreversible inactivation functions of the parent linear peptides (PTs). We also showed that the proteolytic susceptibility of cPTs was massively reduced compared to that of the corresponding linear PTs. Here, we have followed up with structural optimization and minimization to produce a next generation of cPTs. We identified potent cPT analogues by replacing the Trp and Ile residues in the pharmacophore of first generation cPTs. To increase the drug-like nature of cPTs, we aimed to replace the ferrocene moiety through examination of different substituents at the triazole ring. This led to identification of smaller aromatic rings with similar potencies compared to the parent ferrocene-containing cPTs. We also identified smaller 5-residue cPTs that were weaker but still retained the dual host cell receptor binding antagonism properties of the 6-residue cPTs. We further evaluated the binding of both linear (UM15) and cPT (AARO29b) to two structurally defined HIV-1 trimeric protein constructs; i) BG505 SOSIP.664 gp140 (PDB:4NCO) - a highly stable variant that resembles native virus spikes in binding to CD4 receptor as well as known conformationally-dependent gp120 antibodies and ii) JRFL Env(-)ΔCT (EMD-5447) – a heavily glycosylated envelope trimer in its uncleaved and unliganded state. These optimization/minimization steps have led to understanding key structural requirements for PT activities, in general. In turn, this understanding is being used to identify smaller non-peptide scaffolds that could lead to third generation cPT-derived HIV-1 inhibitors.

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T23. Structure-Based Optimization of Small-Molecule CD4-Mimics: Inhibitors of HIV-1 Entry

Sharon M. Kirk, Melissa C. Grenier, Althea E. Gaffney, Bruno Melillo, Amos B. Smith, III

Department of Chemistry, University of Pennsylvania

With approximately 36.9 million people living with HIV worldwide and an additional 2.0 million new infections reported each year, the need to derive novel strategies for the prevention of transmission and suppression of viral reservoirs remains of critical importance. Our program aims to develop potent small-molecule inhibitors of HIV-1 cellular entry based on our growing understanding of the structural and functional mechanism of the HIV-1 envelope glycoprotein (Env) complex. Towards this objective, structure-based optimization has led to the development of potent and broadly active small-molecule CD4 mimics (exemplified by (+)-(R,R)-BNM-IV-147), which upon Env binding can elicit an immune response in HIV-1 infected individuals. Continued synthetic efforts around the indane scaffold and critical oxalamide linker look to further increase the therapeutic potential of these small molecules, aimed at specifically targeting HIV-1 infected cells.

T24. DeNovo Design and Refinement of Inhibitors Targeting HIV Entry

Brian C. Fochtman[1] William J. Allen[2] and Robert C. Rizzo[2,3,4]

[1]Department of Biochemistry and Cellular Biology, [2]Department of Applied Mathematics & Statistics, [3]Institute of Chemical Biology & Drug Discovery, [4]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

In this work we demonstrate the potential utility of a DeNovo platform we have added to the DOCK6 small ligand modelling program. DeNovo small molecule design uses fragments to computationally generate small molecules specifically tailored to a given target and offers a promising alternative to virtual screening. Additionally, such algorithms can provide novel hypotheses during the lead refinement process by sampling fragments at user-defined "R" group on known active scaffolds. As validation, we explore the ability of DeNovo DOCK to rebuild a testset of 663 small molecule – protein complexes taken from the SB2012 testset under various conditions. When using restricted fragment libraries we are able to rebuild a high percentage of co-crystallized ligands in their cognate receptor in a "native-like" pose. For larger, generic fragment libraries we are able to build ensembles of molecules that score well in footprint, grid, or pharmacophore spaces. Examples of DeNovo DOCK being used to target HIVgp41 through both denovo design and refinement are also presented.

T25. HIV-1 Lytic Inactivation by Dual Acting Virus Entry Inhibitor Occurs Through Combined Interactions with gp120 and gp41 Subunits of Virus Env Protein Trimer

Bibek Parajuli[1], Kriti Acharya[1], Reina Yu[1], Adel A. Rashad[1], Cameron F. Abrams[2], Irwin M. Chaiken[1*]

[1]Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, Pennsylvania 19102, United States; [2]Department of Chemical and Biological Engineering, Drexel University, Philadelphia, Pennsylvania 19104, United States

We recently reported discovery of a recombinant chimera, denoted DAVEI (Dual Acting Virus Entry Inhibitor), which is able to selectively cause specific and potent lytic inactivation of both pseudotyped and fully infectious HIV-1 virions. The chimera is composed of the lectin cyanovirin-N (CVN) fused to the 20-residue membrane-proximal external region (MPER) of HIV-1 gp41. Since the Env gp120-binding CVN domain on its own is not lytic, we sought here to determine how the $MPER_{(DAVEI)}$ domain is able to endow the chimera with virolytic activity. We used a protein engineering strategy to identify molecular determinants of $MPER_{(DAVEI)}$ important for function. Recombinant mutagenesis and truncation demonstrated that the $MPER_{(DAVEI)}$ domain could be significantly minimized without loss of function. Dependence of lysis on specific MPER sequences of DAVEI, determination of minimal linker length, and competition by a simplified MPER surrogate peptide suggested that the MPER domain of DAVEI interacts with the Env spike trimer, likely with the gp41 region. This conclusion was supported by observation of binding of biotinylated MPER surrogate peptide to Env protein expressed on cells and by comparison of maximum inter-domain spacing in DAVEI to high-resolution structures of Env. The finding that $MPER_{(DAVEI)}$ in CVN-MPER linker sequences can be minimized without loss of virolytic function provides an improved experimental path to construct size-minimized DAVEI chimeras and molecular tools to determine how simultaneous engagement of gp120 and gp41 by these chimeras can disrupt the metastable virus Env spike.

T26. Design, Synthesis, and Biological Evaluation of Helical Spiroligomers Targeting HIV-1 Gp41

Cheong, J. E. and Schafmeister, C. E.

Department of Chemistry, Temple University

Spiroligomers, chains of bis-amino acids monomers coupled through pairs of amide bonds, are shape-programmable molecules that can be used to control the length, direction, and shape of the scaffold. These *trans*-4-hydroxy-*L*-proline derived molecules can be diversely functionalized and connected for site-specific interactions such as catalysis and molecular recognition applications. We have previously demonstrated that spiroligomers are capable of mimicking the α-helices to be potent inhibitors of protein-protein interaction that binds and stabilizes the target protein (Mdm2) *in vivo*. In this study, new class of spiroligomers has been developed through C-2 alkylation for access to all possible diastereomers. With these the highly functionalized spiroligomers, potential therapeutics for targeting the envelope transmembrane protein gp41 in HIV-1 was designed and implemented as small molecule for biological evaluation to inhibit the gp41-mediated HIV entry process. Infection inhibition is in the process of being determined.

T27. Molecular Dynamics Simulations of HIVgp41 Reveal Energetically Favorable Interfaces for Small-Molecule Inhibitors

T. Dwight McGee Jr.[1], and Robert Rizzo[1,2,3]

[1]Department of Applied Mathematics & Statistics, [2]Institute of Chemical Biology & Drug Discovery, [3]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

Human Immunodeficiency Virus gp41 (HIVgp41) plays an essential role in viral entry and is an attractive drug target. In this work, Molecular Dynamics simulations of the FDA-approved peptide inhibitor Fuzeon (T20) complexed with the N heptad repeat trimer (NHR) of HIVgp41 embedded in an explicit DOPC membrane were performed to identify regions along the NHR interface that could interact energetically favorably with small molecules. The time-averaged van der Waals and electrostatic interactions between T20 and HIVgp41 were computed revealing a putative site termed LDWAW, which are the residues of T20 that interpolate a pocket proximal to the lipid interface. As a proof-of-principle, a preliminary virtual screen of ca. 100K ligands from the commercially available ZINC database to this site suggest that this region can be employed to prioritize potential small-molecule drug leads for subsequent experimental testing to arrest viral entry.

T28. Expression of HERV-K108 Envelope Interferes with HIV-1 Production

Sandra Terry[1], Alvaro Cuesta-Dominguez[1], Lara Manganaro[1], Daria Brinzevich[1], Viviana Simon[1,2], and Lubbertus C.F. Mulder[1, 2]

[1]Department of Microbiology, [2]Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY

The HML-2 group of HERV-Ks includes retroviruses that have entered the germ-line relatively late during human evolution. Such characteristic makes them more likely to have maintained intact open reading frames with the ability to express functional proteins. Approximately 90 full-length or near full-length HML-2 HERV-Ks have recently been identified and their genomic localization has been established. Expression of HERV-Ks has been linked to different pathological conditions, including HIV infection. HERV-K108 is one of the best-studied HERV-Ks, it retains all open reading frames intact and its expression is often found to be prevalent in transformed tissues such as melanomas. Given that endogenous retroviral proteins were among the first restriction factors discovered, we asked whether HERV-K envelopes could have a measurable influence on any of HIV-1 replication steps. In this study we observe that the expression of the envelope of certain HERV-Ks has a negative effect on HIV production. The interference activity was limited to type-2 HERV-Ks and absent in type-1. The inhibition is not a general phenomenon as GFP and Ebola VP40 expression were not affected. While all the HIV-1 strains tested were sensitive to HERV-K-108 inhibition, SIVs exhibited various degrees of sensitivity. Using site directed mutagenesis, we identify four specific residues within HERV-K108 Env as being responsible for the HIV-1 production inhibition. Indeed, changing the identity of all four residues within the HERV-K envelope rescued HIV-1 production. These findings demonstrate HERV Envs can limit HIV-1 viral production. Further studies will be needed to investigate the cellular circumstances under which HERV Envs are expressed, and how harnessing these protective elements may interfere with HIV-1 life cycle.

T29. Minor Sequence Differences in HIV-1_{NL4-3} and HIV-1_{LAI} Capsid Cause Distinct Capsid Uncoating and Host Cell Infectivity Phenotypes

Douglas K. Fischer[1,2,4], Simon C. Watkins[3,4], Masahiro Yamashita[4,5], Tatyana Polenova[4,6], and Zandrea Ambrose[1,2,4]

[1]Molecular Virology and Microbiology Program, [2]Division of Infectious Diseases, Department of Medicine, [3]Department of Cell Biology and Physiology, and [4]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA; [5]Aaron Diamond AIDS Research Center, New York, NY; and [6]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE

The continued successful treatment of individuals infected with human immunodeficiency virus type 1 (HIV-1) requires the discovery of novel antiviral targets to counter the ongoing development of drug resistance. After host cell entry, HIV-1 reverse transcribes its RNA genome into double-stranded DNA, which is then translocated to the nucleus for integration. A key aspect of this HIV-1 life cycle stage is the dissociation of the viral capsid protein, which is cleaved from the viral Gag polyprotein and shields the viral genome, in a tightly regulated process called uncoating. Our lab developed a fluorescence assay to visualize and examine the kinetics of HIV-1 capsid uncoating within infected cells. This assay stains 5-ethynyl uridine (EU) incorporated into the viral RNA genome with an EU-specific fluorescent dye that requires opening of the capsid. The fluorescent signal shows the initiation of uncoating after HIV-1 cell entry and is colocalized with fluorescently tagged integrase packaged into the virus *in trans*. This assay revealed that common lab-adapted HIV-1 strains NL4-3 and LAI exhibit different uncoating kinetics in a Gag-dependent manner. An examination of HIV-1 capsid mutants with demonstrated cell cycle and/or Cyclosporine A dependence and impaired host factor interactions further showed that the two HIV-1 strains have different Gag-dependent infectivity phenotypes with these capsid mutants in multiple human cell lines and in primary human CD4+ T cells. Additional data suggests the four amino acids that differ between HIV-1_{NL4-3} and HIV-1_{LAI} capsid play an integral role in these phenotypes. Work is ongoing to characterize the specific capsid amino acid differences between these HIV-1 strains that are responsible for these changes in uncoating and infectivity, and to determine the mechanism(s) through which these distinct phenotypes are produced. Studies are also underway to examine potential structural alterations in assembled capsid by solid-state NMR. A better understanding of how different HIV-1 strains interact with host cell processes can provide valuable insight into aspects of the virus life cycle that might be targeted by novel therapeutics.

T30. Real-Time Imaging of Single HIV-1 Uncoating in Cells

Ashwanth C. Francis[1], Mariana Marin[1], Jiong Shi[2], Christopher Aiken[2] and Gregory B. Melikyan[1,3]

[1]Department of Pediatric, Emory University School of Medicine, Atlanta, GA; [2]Department of Pathology, Microbiology and Immunology, Vanderbilt University, Nashville, TN; [3]Children's Healthcare of Atlanta, Atlanta, GA

HIV-1 uncoating, a necessary step in infection, which involves shedding of the capsid protein from the core complex encasing the viral genome, is poorly understood. To elucidate this critical step of HVI-1 entry, we developed a novel strategy to visualize HIV-1 uncoating using a fluorescently tagged oligomeric form of a capsid-binding host protein cyclophilin A (CypA-DsRed), which is specifically packaged into virions through the high-avidity binding to capsid (CA). Single virus imaging reveals that CypA-DsRed remains associated with cores after permeabilization/removal of the viral membrane. Importantly, CypA-DsRed and CA are lost concomitantly from the cores *in vitro* and in living cells. The rate of CypA-DsRed loss is modulated by the core stability and is accelerated upon the initiation of reverse transcription. The majority of single cores lose CypA-DsRed shortly after viral fusion, while a small fraction remains intact for several hours. Single particle tracking at late times post-infection reveals a gradual loss of CypA-DsRed which is dependent on reverse transcription. Uncoating occurs both in the cytoplasm and at the nuclear membrane. To conclude, the novel CypA-DsRed based imaging assay enables time-resolved visualization of single HIV-1 uncoating in living cells and provides important clues regarding its spatio-temporal regulation.

T31. Correlation of Infectivity and Imaged Individual HIV Particle Behavior Validates the Early Uncoating Model During HIV Infection

João I. Mamede, Gianguido C. Cianci, Meegan Anderson, Thomas J Hope

Northwestern University Feinberg School of Medicine, Department of Cell and Molecular Biology, Chicago, IL

Individual viral particle tracking and analysis has become widely utilized as a tool to study the cell biology of HIV replication. Although this approach has advanced the field, it has faced the important criticism that it is not possible to filter the observations to focus on the particles that ultimately infect a cell. Utilizing variable time lapse imaging of multiple fields of cells over periods up to 36 hours allows the identification of productively infected cells as visualized by reporter virus expression. Infection with very low MOI allows conditions where there is less than one labeled particle per cell. In this way correlation of individual particle behavior can be directly connected to infection. We have recently developed the use of intravirion fluid phase markers to determine the integrity of the HIV conical capsid core. To visualize dynamic changes in capsid integrity and composition, we utilized the HIV-iGFP construct. During viral maturation of HIV-iGFP, the GFP is liberated from Gag; a minority population of the free GFP is trapped in the capsid, while the remaining free GFP is located outside of the capsid. With this technique, the loss of the fluid phase GFP occurs in two steps, with fusion and upon the loss of capsid core integrity. Live-cell microscopy of HIV-iGFP virions with a second color labeled viral core protein such as Vpr or Integrase allows for the timing of these two steps thereby revealing the kinetics, localization and composition of HIV-1 early steps of infection.

The timing of uncoating remains under discussion with some models proposing that uncoating happens early. Other models suggest that the intact capsid docks at the nuclear pore thereby protecting the viral genome from detection by innate sensors in the cytoplasm. The differences of these models combined with the wide assay variability and the lack of data for the kinetics and localization of uncoating, has led to debate over recent years. Our data reveals that the time between fusion and capsid integrity loss, for both HIV and VSV-G mediated fusion, in tissue culture and primary cells (macrophages and T cells), is approximately 25 minutes. Also, capsid integrity loss occurs entirely in the cytoplasm. However, we also detect a second species, which does not experience a change in capsid integrity over 2 hours of imaging. Through viral challenge with less than one particle per cell over a long period of time, we were able to image individual particle capsid integrity loss that produces a viable infection. This analysis revealed that all particles associated with cellular infection showed changes in capsid integrity ~25 minutes. Together, these observations validate the early cytoplasmic uncoating model. The ability to follow uncoating at the single infectious particle level opens up many opportunities to define and characterize the earliest steps of the HIV lifecycle and how the virus interacts with innate host defenses.

T32. Development of Cryo-CLEM Methods to Elucidate the Intracellular Structure of TRIM5α Bodies

Stephen D. Carter[1], Shrawan K. Mageswaran[1], Joao I. Mamede[3], Tom J. Hope[3], Joachim Frank[4,5], Zachary Freyberg[6,7], and Grant J. Jensen[1,2]

[1]Division of Biology and [2]Howard Hughes Medical Institute (HHMI), California Institute of Technology, Pasadena, CA 91125, USA; [3]Department of Cell and Molecular Biology, Northwestern University, Chicago, IL 60611-3008; [4]HHMI; Department of Biochemistry and Molecular Biophysics, Columbia University, New York, New York 10032, USA; [5]Department of Biological Sciences, Columbia University, New York, New York 10027, USA; [6]Department of Psychiatry, College of Physicians & Surgeons, Columbia University, New York, New York 10032, USA; [7]Division of Molecular Therapeutics, New York State Psychiatric Institute, New York, New York 10032, USA

The mammalian TRIM5α protein is a host restriction factor that inhibits retroviral replication in a species-dependent manner. HIV-1 restriction by rhesus (rh) TRIM5α prevents the viral pre-integration complex from entering the nucleus by binding and targeting the HIV-1 capsid at multiple stages in the cytoplasm. The exact mechanism by which rh-TRIM5α restriction occurs is uncertain, but *in vivo* live fluorescence analysis has suggested the importance of dynamic TRIM5α bodies. To understand the role of TRIM5α bodies in HIV-1 restriction we developed cryogenic correlated light microcopy and electron microscopy (cryo-CLEM) methods to visualize cytoplasmic TRIM5α-YFP bodies in HeLa cells. This procedure allows for the visualization of localized fluorescently-tagged proteins down to the level of individual organelles. However, during the methods development process, we discovered in frozen cells that there are many bright sources of autofluorescence. Hypothesizing that autofuorescence would exhibit a broader spectrum of fluorescence than fluorescence from fluorescent proteins, we developed a quantitative approach to discriminate between autofluorescence and real fluorescent protein signal by looking at both fluorescent intensities across different channels. To validate this new methodology, we targeted a number of fluorophore-tagged organelle protein markers including DSRed2-Mito and chromogranin A-GFP in order to specifically label and image already known structures, such as secretory granules and mitochondria.

T33. Characterization of TRIM5 Assembly and Activation Using Chimeric "MiniTRIMs"

Jonathan M. Wagner[1], Marcin D. Roganowicz[1], Katarzyna Skorupka[1], Steven L. Alam[2], Devin Christensen[2], Ginna Doss[1], Yueping Wan[1], Gabriel A. Frank[3], Barbie K. Ganser-Pornillos[1], Wesley I. Sundquist[2], and Owen Pornillos[1]

[1]Department of Molecular Physiology and Biological Physics, [2]Department of Biochemistry, University of Utah, Salt Lake City, UT 84112, U.S.A.; [3]Faculty of Biology, Technion - Israel Institute of Technology, Haifa 320003, Israel

TRIM5α is a retroviral restriction factor that is able to intercept incoming HIV capsids and thereby accelerate uncoating and disrupt reverse transcription. In addition, TRIM5α acts as a cytosolic pattern recognition receptor that activates the cell's anti-viral response via ubiquitin-mediated interferon signaling. The first critical step for TRIM5α function is capsid recognition. TRIM5α dimers must self assemble on the surface of capsids, creating a hexagonal network that positions capsid binding domains over repeating epitopes on the capsid surface leading to highly avid binding. The current model of TRIM5 self assembly presumes that trimerization of the B-box domains is responsible for higher order oligomerization. B-box trimerization also brings their associated RING domains into close proximity, resulting in activation of the RING domain and ubiquitin transfer. Structural confirmation of B-box trimer formation has been very challenging due to the difficulty of purifying and crystallizing full-length TRIM5 proteins. In order to prevent formation of insoluble TRIM5α assemblies during crystallization and biochemical experimentation, we created a chimeric "miniTRIM" construct consisting of the B-box domain and part of the coiled-coil domain dimer. These miniTRIMs were readily purified and crystallized in both dimeric and trimeric oligomeric states. These structures are consistent with the current model of B-box mediated hexagonal TRIM5 lattices, and provide insight into how B-box interactions activate the RING E3 ligase activity for ubiquitin signaling.

T34. Analysis of TRIM5α SPRY Domain Packing Against Its Coiled-Coil Domain

Marcin D. Roganowicz[1], Santanu Mukherjee[2], Katarzyna Skorupka[1], Damian Dawidowski[3], David S. Cafiso[3], Edward M. Campbell[2], Owen Pornillos[1]

[1]Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA; [2]Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, Maywood, IL; [3]Department of Chemistry, University of Virginia, Charlottesville, VA

The rhesus TRIM5α protein is a cytosolic restriction factor that inhibits various retroviruses, including HIV-1, and is one of the best studied members of RBCC family. It consists of an N-terminal RING domain harboring E3 ligase activity, crucial for inhibition of reverse transcription; a B-box 2 domain responsible for high-order oligomerization; a coiled-coil domain that mediates homodimerization by formation of antiparallel hairpin structure; and a C-terminal SPRY (or B30.2) effector domain that is pivotal for direct binding to HIV-1 capsid and thus viral restriction. The coiled-coil domain is linked to the SPRY domain via the L2 linker. We and others have proposed that the terminal L2 helices pack against the central regions of the coiled-coil dimer in order to position the SPRY domains for optimal capsid recognition and viral restriction. By computationally combining available structures of the B-Box 2/coiled-coil dimer and the isolated SPRY domain, we predicted two classes of residues that are responsible for either coiled-coil dimerization or take part in packing of L2 helices against coiled-coil. Alanine screening of these side chains revealed impaired capsid binding, inhibited restriction activity, and sometimes lowered protein stability. Additionally, the mutants producing defects in coiled-coil dimerization were impaired in cytoplasmic body formation. In future studies, we will use electron paramagnetic resonance experiments to investigate the mobility of the L2 helices and SPRY domains, both for the protein alone and protein bound to capsid-mimicking CA tubes. Initial studies revealed that the L2 helices are highly mobile, when no SPRY domain is present.

T35. Dynamic Allostery in HIV-1 Capsid Interactions with Restriction Factor TRIM5 Revealed by Magic Angle Spinning NMR

Caitlin M Quinn[1,2], Mingzhang Wang[1,2], Jinwoo Ahn[2,3], Angela Gronenborn[2,3], and Tatyana Polenova[1,2]

[1]University of Delaware, Department of Chemistry and Biochemistry, Newark, DE; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 5th Ave., Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA

The restriction factor protein TRIM5α (tripartite motif isoform 5α) is a powerful retroviral inhibitor with species-specific potency against HIV.[1] Upon fusion of the viral particle with the host cell plasma membrane, TRIM5α targets the retroviral capsid (CA) and induces premature disassembly of the capsid thus inhibiting retroviral replication.[2] While biochemical and structural studies have lent insight into TRIM5α-capsid interactions, atomic-level characterization of TRIM5α interactions with the assembled capsid has not been possible to date. Magic angle spinning (MAS) NMR is uniquely suited to access this information and has provided atomic-resolution structural and dynamics information in several HIV-1 protein assemblies as shown by us and by others.[3] We present direct, residue-specific evidence of intermolecular interactions between the CC-SPRY (coiled-coil and PRY/SPRY) domain of TRIM5α and tubular assemblies of cross-linked hexameric CA. We demonstrate that the capsid undergoes numerous structural perturbations upon binding of TRIM5α, including conformational and dynamic changes both at the binding interface and distal to this interface, as evidenced by spectral changes including chemical shift perturbations, changes in linewidths, and the appearance or disappearance of multiple peaks for several residues. The binding of TRIM5α results in global attenuation of capsid dynamics. We further observe perturbations at the intermolecular interfaces identified as being essential for capsid stability and viral infectivity. The fact that structural and dynamic changes occur throughout the entire CA protein as the result of TRIM5α binding, rather than being limited to the protein-protein interface, suggests that TRIM5α employs several mechanisms to promote instability of the capsid lattice, and ultimately induce its premature disassembly. This work was supported by the National Institutes of Health (P50GM082251 and F32GM113452) and is a contribution from the Pittsburgh Center for HIV Protein Interactions. We acknowledge the support of the NSF CHE0959496 grant for acquisition of the 850 MHz NMR spectrometer and of the NIH P30GM110758-01, P30GM103519 grant for the support of core instrumentation infrastructure at the University of Delaware.

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T36. Unbiased Genome Wide Screens for Host Cofactors Involved in Viral Restriction

Daniel W. Cyburt, Clifton L. Ricana, and Marc C. Johnson

Department of Molecular Microbiology and Immunology, University of Missouri-Columbia

TRIM5 and Fv1 are retroviral restriction factors that block infection early in the viral lifecycle soon after viral entry. TRIM5α from rhesus monkey (rhTRIM5α) restricts human immunodeficiency virus (HIV-1), human TRIM5 (huTRIM5α) restricts N-tropic murine leukemia virus (MLV) but not B-tropic MLV, and N-tropic Friend virus susceptibility factor-1 ($Fv1^N$) restricts B-tropic MLV but not N-tropic MLV. These blocks are all saturable and occur post-fusion but prior to integration. Interestingly, it has been reported that some of these restriction factors exhibit variable levels of viral restriction depending on the cell line they are introduced into. This suggests that these restriction factors may utilize cellular cofactors during retroviral restriction that are present in some cell lines but absent in others. Previous studies have identified cofactors for TRIM5, but none of these have since been shown to be required for restriction. To identify potential cellular cofactors required for restriction, we are performing a genome-wide screen using the human and murine versions of the CRISPR/Cas9 GeCKO library. These libraries were used to target 19,052 human and 20,661 murine genes in 293FT cells expressing huTRIM5α or rhTRIM5α, or N-tropic murine cells. These CRISPR cell library populations are challenged with restricted virus carrying a selectable marker. Antibiotic resistance is used to select for cells from the resistant population that have become sensitive to infection due to knockout of a critical cofactor. After each round, the selected populations are tested for enhanced viral susceptibility. Once resistant populations have lost their resistance, genomic DNA from these selected cells will be isolated and the CRISPR guide RNA sequence will be identified. This sequence will point to gene knockouts associated with loss of restriction. Each of these candidate cofactor knockouts will be validated individually, outside the context of the genome-wide screen.

Use of this non-biased approach, coupled with an infectivity-based screening mechanism, should allow a wider search than has been previously possible, and the added ability to focus only on required cofactors for retroviral restriction.

T37. Investigation of NMR Spectral Changes upon Homodimer Formation of HIV-1 Reverse Transcriptase

Ryan L. Slack[1], Naima G. Sharaf[1], Michael A. Parniak[2], Jinwoo Ahn[1], Angela M. Gronenborn[1], Rieko Ishima[1]

[1]Department of Structural Biology and [2]Department of Microbiology and Molecular Genetics, University of Pittsburgh, School of Medicine, PA-15260, USA

Experimental NMR data obtained for large proteins in solution often suffer from line-broadening, resonance overlap and signal losses. To address these practical challenges, labeling schemes and optimized pulse sequences are used to enhance sensitivity of the data acquired; and designed assignment strategies are used to improve experimental tractability. We have used these approaches to characterize changes in HIV-1 Reverse Transcriptase (RT), the multidomain enzyme responsible for the viral DNA polymerase and ribonuclease activities required for replication.

RT is encoded as a 66 kDa polypeptide in the Gag-Pol polyprotein and matured to form a heterodimer composed of 66 kDa (p66) and 51 kDa (p51) subunits. The p51 subunit is generated by proteolytic cleavage of the C-terminal ribonuclease H domain (RNH) from a p66 protein (1-6). Evidence suggests that this proteolytic maturation step proceeds from an immature p66 homodimer (7-10), however little structural information has been reported for this homodimeric intermediate.

We previously established that the overall structures of the Thumb and RNH domains within p66 are very similar to those of the isolated domains in solution (11). Building upon this work, we preformed TROSY-HSQC experiments of [^{2}H,^{15}N] labelled samples to characterize the homodimer interface, as well as the residues and domains affected by p66 homodimerization. Additionally, HMQC experiments of [methyl-^{13}C] Methionine labeled samples were conducted to investigate the effect of buffer ionic strength on subunit dimerization. This coupling of labeling schemes with experimental selection and designed assignment strategy make elucidation of structural information feasible for large proteins in solution. (Supported by: the National Institutes of Health P50GM082251 and R01GM105401)

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T38. Biochemical and Cellular Characterization of the SAMHD1 Ortholog, *Caenorhabditis Elegans* ZCK177.8

Lydia R. Studdard, Tatsuya Maehigashi and Baek Kim

Center for Drug Discovery, Emory School of Medicine, Pediatrics, Atlanta, GA

SAM domain and HD domain containing protein 1 (SAMHD1) was identified as a myeloid specific host restriction factor against HIV-1. The dNTP triphosphohydrolase (dNTPase) activity of SAMHD1 limits dNTP levels particularly in macrophages and non-dividing cells by degrading dNTPs into deoxynucleosides and triphosphates, which in turn suppresses viral reverse transcription kinetically. Recently, its involvement in a neuro-developmental genetic disorder, Aicardi Goutieres Syndrome (AGS) has been indicated, as several mutations in human SAMHD1 gene were identified in AGS patients. *Caenorhabditis elegans* (*C. elegans*) encodes a SAMHD1 ortholog, ZK177.8, which reportedly also induces developmental defects upon the gene knockdown. Here we demonstrate that ZK177.8 is also an allosterically-regulated dNTPase. ZK177.8 is predominantly found as a dimer in a solution; however incubation with increasing amount of dGTP induced the formation of a higher oligomeric state (tetramer). Its dNTPase activity was also only observed upon incubation with dGTP or GTP as seen in human SAMHD1. ZK177.8 recognized both 2' OH and triphosphate of the dNTP substrates for the dNTP hydrolysis as it's unable to degrade NTPs or various nucleotide chain terminators. Finally, we examined the ability of ZK177.8 to lower the cellular dNTP levels and restrict HIV-1 in a human macrophage cell line lacking SAMHD1. In conclusion, this study suggests that *C. elegans* ZK177.8 can serve as a model for the SAMHD1-mediated AGS.

T39. Effect of Nucleic Acid Sequence on DNA Polymerization and NNRTI Inhibitory Mechanisms of HIV-1 Reverse Transcriptase

O Ukah[1], A Huber[2], E Michailidis[3], K Das[4], MA Parniak[5], K Singh[1], E Arnold[4], and S Sarafianos[1,6]

[1]University of Missouri, Molecular Microbiology and Immunology, Columbia, MO, United States; [2]University of Missouri, Veterinary Pathobiology, Columbia, MO, United States; [3]Rockefeller University, Virology and Infectious Disease, New York, NY, United States; [4]Rutgers University, Chemistry and Chemical Biology, Piscataway, NJ, United States; [5]University of Pittsburgh, Microbiology and Molecular Genetics, Pittsburgh, PA, United States; [6]University of Missouri, Biochemistry, Columbia, MO

HIV-1 reverse transcriptase (RT) is a well-studied enzyme that is the target of nonnucleoside RT inhibitors (NNRTIs), a class of antiviral agents used to treat HIV infection. Numerous biochemical and structural studies have provided valuable insights into the mechanisms by which NNRTIs affect the activity of HIV RT. Structural comparisons of RT in complex with various NNRTIs have revealed differences in the conformation of DNA-interacting structural regions of RT, such as the p66 thumb and the DNA primer grip. Moreover, a recent crystal structure of RT in complex with DNA and nevirapine (Das *et al.* NSMB, 19 253-9) has shown that NNRTI binding distorts the dNTP-binding site that comprises both protein and DNA components, and also repositions the 3' end of the DNA primer. Hence, we hypothesized that variations in DNA sequence can modulate the efficacy of NNRTIs and their mechanism of inhibition.

To test this hypothesis, we determined the position of RT on DNA of various sequences using a site-specific hydroxyl radical foot printing assay in the presence and absence of different concentrations of various NNRTIs. This approach revealed surprising changes in RT-DNA binding with lateral displacements of template/primers that were dependent on the nucleic acid sequences, as well as on the type on NNRTI bound. To determine whether these site-specific changes correlate with differences in the ability of RT to biochemically recognize the dNTP and DNA substrates, we performed transient-state kinetic analysis and demonstrated significant sequence-specific changes in DNA- and dNTP-binding affinities ($K_{d.DNA}$ and $K_{d.dNTP}$ respectively) and catalytic turnover (k_{pol}).

Taken together, our data suggest that (a) NNRTIs have significant effects on RT-DNA binding conformations, which are greatly influenced by the DNA sequence and the type of NNRTI, and that (b) the DNA polymerization properties of RT vary significantly with DNA sequence.

T40. HIV-1 Capsid Facilitates Reverse Transcription by Retaining Reverse Transcriptase Within the Core

Janani Varadarajan and Christopher Aiken

Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232

During entry into target cells, the human immunodeficiency virus 1 (HIV-1) releases the viral capsid harboring its RNA genome into the cytoplasm. A productive HIV-1 infection involves two critical early steps: disassembly or “uncoating” of the capsid and conversion of the single-stranded RNA genome into double-stranded DNA by the viral reverse transcriptase (RT). Mutations altering the intrinsic stability of the viral capsid frequently result in impaired infectivity and viral DNA synthesis is impaired in viruses with unstable cores. This indicates that uncoating is a temporally-regulated process ensuring efficient reverse transcription. The HIV-1 RT is a low processivity enzyme that pauses and dissociates frequently from the RNA and DNA templates during viral DNA synthesis. Furthermore, we have observed that RT dissociates from the core at the same rate as the capsid protein during uncoating of purified HIV-1 cores *in vitro*. Based on these observations, I hypothesized that the viral capsid retains RT within the core to ensure completion of reverse transcription prior to undergoing uncoating. To test this hypothesis, we performed biochemical analysis of HIV-1 cores isolated from wild type (WT) and mutant cores containing unstable, and hyperstable capsids, and performed functional assays of reverse transcription *in vitro*. Relative to the WT, cores with unstable capsids harbored reduced levels of RT, whereas hyperstable cores contained elevated levels of RT. This suggests that RT is released from the unstable cores prematurely. Further, presence of capsid destabilizing antiviral compound resulted in impaired *in vitro* reverse transcription in isolated WT cores but not in hyperstable cores. These results indicate that the integrity of the viral capsid is critical for the completion of viral DNA synthesis. The correlation between the capsid stability, RT levels and reverse transcription efficiency supports the hypothesis that the viral capsid serves as a container that retains RT within the core to ensure completion of reverse transcription.

T41. 3-Hydroxypyrimidine-2,4-diones as Novel HIV-1 RNase H Inhibitors

Karen A. Kirby[1,2], Jing Tang[3], Sanjeev K. V. Vernekar[3], Bulan Wu[3], Andrew D. Huber[1,4], Mary C. Casey[1,2], Juan Ji[1,2], Eva Nagy[5], Lena Miller[5], Qiongying Yang[1,2], Michael A. Parniak[5], Zhengqiang Wang[3], and Stefan G. Sarafianos[1,2,6]

[1]C.S. Bond Life Sciences Center, University of Missouri, Columbia, MO; [2]Dept. of Molecular Microbiology & Immunology, University of Missouri School of Medicine, Columbia, MO; [3]Center for Drug Design, University of Minnesota, Minneapolis, MN; [4]Dept. of Veterinary Pathobiology, University of Missouri School of Medicine, Columbia, MO; [5]Dept. of Microbiology & Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA; [6]Dept. of Biochemistry, University of Missouri, Columbia, MO

The reverse transcriptase (RT) enzyme of HIV-1 plays a critical role in viral replication, using polymerase and RNase H (RNH) functions to convert the single-stranded RNA viral genome into double-stranded DNA for subsequent integration into the host genome. All currently FDA-approved drugs against RT only target the polymerase function of this enzyme. The RNH activity of RT is the last enzymatic function of HIV-1 yet to be targeted by approved therapies and is therefore an attractive antiviral target.

Our team is using a structure-based design approach to improve existing leads and develop new RNH inhibitors (RNHIs) with increased potency. We have synthesized several compounds based on the 3-hydroxypyrimidine-2,4-dione (HPD) scaffold. Although several analogues demonstrated mid-nanomolar (150-900 nM) potency in RT-associated RNH inhibition assays, none were able to inhibit HIV-1 in cell-based assays. Further design of HPD analogues resulted in compounds with low nanomolar (50 nM) potency in RT-associated RNH assays and one compound with low micromolar efficacy in cell-based assays. This lead compound does not inhibit integrase in *in vitro* strand transfer assays. Crystal structures of several HPD analogues in complex with RT provide insights into the molecular details of RNH inhibition. Information gained from these structures should help guide design efforts based on these scaffolds that will potentially give rise to new inhibitors with enhanced antiviral potency.

T42. Two Distinct Modes of Metal Ion Binding in the Nuclease Active Site of a Viral DNA-Packaging Terminase: Insight into the Two-Metal-Ion Catalytic Mechanism of RNaseH-like Nucleotidyltransferases

Haiyan Zhao[1], Zihan Lin[1], Anna Y. Lynn[1], Brittany Varnado[1], John A. Beutler[2], Ryan P. Murelli[3], Stuart F.J. Le Grice[4] and Liang Tang[1]

[1]Department of Molecular Biosciences, University of Kansas, 1200 Sunnyside Avenue, Lawrence, KS 66045; [2]Molecular Targets Laboratory, National Cancer Institute, Frederick, MD 21702; [3]Department of Chemistry, Brooklyn College, City University of New York, Brooklyn, NY 11210; [4]Basic Research Laboratory, National Cancer Institute, Frederick, MD 21702

Many double-stranded DNA viruses, including those of herpesviruses, encode DNA-packaging molecular machines, designated, terminases, whose nuclease domain resolves concatemeric DNA into genome-length units. The terminase catalytic subunit comprises an N-terminal ATPase domain that powers DNA translocation by translating chemical energy from ATP hydrolysis into physical movement of DNA, and a C-terminal nuclease domain that processes the DNA. Terminase nucleases belong to the superfamily of nucleotidyltransferases, members of which include RNases H, reverse transcriptases, integrases, topoisomerases, DNA and RNA polymerases, transposases, Holliday-junction resolvases, RNAi slicer Argonaute and CRISPR Cas nucleases, and are fundamental to an array of biological processes ranging from DNA replication, recombination and repair, RNA maturation, processing and interference, to host defense, cell death and virus genome packaging.

RNase H-like nucleases share a common 2-metal-ion catalytic mechanism where the two metal ions are jointly coordinated by the scissile phosphate and two active-site carboxylates, bisecting the scissile phosphate. Metal ion A is proposed to deprotonate a water molecule to form OH, which is aligned for a nucleophilic attack on the scissile phosphate, while Metal ion B stabilizes the transition state pentacovalent phosphate. However, a detailed catalytic mechanism establishing the precise roles of these metal ions in pentacovalent phosphate formation remains to be established experimentally. The two metal ions were ~3.4-4 Å apart in previous structures of RNase H-like nucleases, and thought to have to move closer, allowing the nucleophile to access the scissile phosphate for phosphodiester bond cleavage. Such a closer metal-metal distance has, however, not been experimentally observed. Our high resolution crystallographic analysis of the bacteriophage Sf6 terminase domain (gp2C) reveals a metal ion binding mode with a unique coupled-octahedral configuration, exhibiting an ultra-short metal-metal distance of 2.42 Å. Such a distance is unusual given the 1.6 Å atomic radius of Mg^{2+} and is among the shortest metal-metal distances in biological systems thus far documented. This provides a structural basis for requirement of Mg^{2+} or Mn^{2+} in RNase H-like nucleases to support catalysis. Such an ultra-short metal-metal distance may generate a highly positive electrostatic niche that drives formation of the pentacovalent phosphate transition state.

T43. Characterization the C-Terminal Nuclease Domain of Herpes Simplex Virus Pul15 as a Target of Nucleotidyltransferase Inhibitors

Takashi Masaoka[1], Haiyan Zhao[2], Danielle R. Hirsch[3,4], Michael P. D'Erasmo[3,4], Christine Meck[3,4], Brittany Varnado[2], Marvin J. Meyers[5] Joel Baines[6], John A. Beutler[7], Ryan P. Murelli[3,4], Liang Tang[2] and Stuart F.J. Le Grice[1]

[1]Basic Research Laboratory, National Cancer Institute, Frederick, MD 21702, USA; [2]Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045, USA; [3]Department of Chemistry, Brooklyn College, City University of New York, Brooklyn, NY 11210, USA; [4]Department of Chemistry, The Graduate Center, City University of New York, New York, NY, 10016, USA; [5]Department of Chemistry, St. Louis University, St. Louis, MO 63103; [6]School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 USA; [7]Molecular Targets Laboratory, National Cancer Institute, Frederick, MD 21702, USA

Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2, respectively) are closely related enveloped alphaherpesviruses with large, double-stranded DNA genomes encoding ~80 proteins. The natural product α-hydroxytropolones manicol and β-thujaplicinol inhibit replication of HSV-1 and HSV-2 at nontoxic concentrations. Because these were originally developed as divalent metal-sequestering inhibitors of the ribonuclease H activity of HIV-1 reverse transcriptase, α-hydroxytropolones likely target related HSV proteins of the nucleotidyltransferase superfamily, which share an "RNase H-like" fold. One potential candidate is the C-terminal nuclease domain of pUL15 (pUL15C), a component of the viral terminase molecular motor complex. Crystallographic analysis and modeling studies of pUL15C suggested a binding cleft that could accommodate ~14 bp of duplex DNA, within which residues Arg517, Arg695, Lys700, and Lys701 were proposed to interact with the phosphate backbone.

In this work, we extended crystallographic analysis by examining pUL15C-mediated hydrolysis of short DNA duplexes and found that a "minimal" 14 bp duplex containing an A:T-rich segment flanked by G:C-rich segments is required for efficient cleavage. In addition to defining a minimal substrate requirement, this strategy facilitated construction of a high-throughput dual-probe fluorescence assay for rapid kinetic analysis of pUL15C. Kinetic studies with pUL15C variant Lys700Ala showed that this mutation affected neither binding of duplex DNA nor binding of α-hydroxytropolone to the active site but caused a 17-fold reduction in the turnover rate (k_{cat}), possibly by slowing conversion of the enzyme−substrate complex to the enzyme−product complex and/or inhibiting dissociation from the hydrolysis product. Finally, with a view of pUL15-associated nuclease activity as an antiviral target, the dual-probe fluorescence assay, in combination with differential scanning fluorimetry, was used to characterize inhibition by three structural classes of 33 small molecules that target divalent metal at the active site. This approach yielded IC_{50} values ranging from 0.12 μM to compound 49.1 μM and showed generally a good correlation between thermal stabilizing effects and IC_{50} values. Further kinetic analysis of pUL15C inhibition revealed that α-hydroxytropolone acts as a non-competitive inhibitor. Our study may provide the foundation for the development and study of a new class of anti-herpes drugs.

T44. Molecular Characterization of a Unique Restriction Factor, APOBEC3H

Jennifer Bohn[1], Theodora Hatziioannou[2], and Janet Smith[1]

[1]Dept. of Biological Chemistry & Life Sciences Institute, University of Michigan, Ann Arbor, MI; [2]Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY

Restriction factors play a critical role in protecting the host from viral infections such as HIV-1. Restriction factors in the APOBEC3 family recognize minus strand (-) DNA (ssDNA) of the HIV-1 genome generated during reverse transcription. This ssDNA is a substrate for zinc-dependent cytidine deamination by APOBEC3 enzymes, resulting in hypermutation of the HIV-1 genome, leading to either degradation of the cDNA or incoherent protein translation. In addition to the cytidine deaminase activity, APOBEC3 proteins bind to host and viral RNAs to facilitate encapsidation into new virions. HIV-1 has adapted mechanisms to counteract these restriction factors. The HIV-1 protein Vif neutralizes restriction activity by associating with APOBEC3 enzymes in order to recruit host ubiquitin ligase machinery to tag APOBEC3 enzymes for proteasomal degradation.

APOBEC3H (A3H) is a unique member of the APOBEC3 family in that it potently restricts HIV-1 infectivity using only a single deaminase domain. We have generated protein expression constructs for several natural polymorphic variants of pig-tailed macaque A3H, which exhibit varying levels of antiviral activity in cells. A3H variants will be recombinantly expressed and purified for crystallographic studies, gel-based deamination activity assays, RNA binding and protein-protein interaction studies. The use of natural polymorphic variants identified in a relevant model system for HIV-1 infection, pig-tailed macaques, will allow us to correlate our biochemical and structural studies of pig-tailed macaque A3H to functionality while characterizing this unique restriction factor on an atomic level.

T45. NMR Structure of the APOBEC3B Catalytic Domain: Structural Basis for Substrate Binding and DNA Deaminase Activity

In-Ja L. Byeon[1], Chang-Hyeock Byeon[1], Tiyun Wu[2], Mithun Mitra[2], Dustin Singer[2], Judith G. Levin[2] and Angela M. Gronenborn[1]

[1]Department of Structural Biology and Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, United States; [2]Section on Viral Gene Regulation, Program in Genomics of Differentiation, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, United States

Human APOBEC3B (A3B) is a member of the APOBEC3 (A3) family of cytidine deaminases, which function as DNA mutators and restrict viral pathogens and endogenous retrotransposons. Recently, A3B was identified as a major source of genetic heterogeneity in several human cancers. Here, we determined the solution NMR structure of the catalytically active C-terminal domain (CTD) of A3B and performed detailed analyses of its deaminase activity. The core of the structure comprises a central five-stranded β-sheet with six surrounding helices, common to all A3 proteins. The structural fold is most similar to that of A3A and A3G-CTD, with the most prominent difference found in loop 1. The catalytic activity of A3B-CTD is ~15-fold less than that of A3A, although both exhibit similar pH dependence. Interestingly, A3B-CTD with an A3A loop 1 substitution had significantly increased deaminase activity, while a single residue change (H29R) in A3A loop 1 reduced A3A activity to the level seen with A3B-CTD. This establishes that loop 1 plays an important role in A3-catalyzed deamination by precisely positioning the deamination-targeted **C** into the active site. Overall, our data provide important insights into the determinants for the activities of individual A3 proteins and facilitate understanding of their biological function.

T46. The RNA Binding Specificity of Human APOBEC3 Proteins Mimics that of HIV-1 Nucleocapsid

Ashley York[1,2*], Sebla B Kutluay[3*], Manel Errando[4], and Paul D Bieniasz[1,2,5]

*[1]Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY; [2]The Rockefeller University, Laboratory of Retrovirology, New York, NY; [3]Washington University School of Medicine in St. Louis, Department of Molecular Microbiology, St. Louis, MO; [4]Department of Physics, Washington University in St. Louis, St. Louis, MO, [5]Howard Hughes Medical Institute, Aaron Diamond AIDS Research Center, New York, NY; *equal contributions*

The APOBEC3 (A3) cytidine deaminases are antiretroviral proteins, whose targets include human immunodeficiency virus type-1 (HIV-1). Their incorporation into viral particles is critical for antiviral activity and is driven by interactions with the RNA molecules that are packaged into virions. However, it is unclear whether or how A3 proteins preferentially target RNA molecules that are destined to be packaged. Using cross-linking immunoprecipitation sequencing (CLIP-seq), we determined the RNA binding preferences of the A3F, A3G and A3H proteins. We found that A3 proteins bind preferentially to RNA segments with particular properties, both in cells and in virions. Specifically, A3 proteins target RNA sequences that are G and/or A-rich and are not scanned by ribosomes during translation. Comparative analyses of HIV-1 Gag, nucleocapsid (NC) and A3 RNA binding to the HIV-1 genome in cells and virions revealed the striking finding that A3 proteins partially mimic the RNA binding specificity of the HIV-1 NC protein. These findings suggest a model for A3 incorporation into HIV-1 virions in which and NC-like RNA binding specificity is determined by nucleotide composition rather than sequence. This model reconciles the promiscuity of A3 RNA binding that has been observed in previous studies with a presumed advantage that would accompany selective binding to RNAs that are destined to be packaged into virions.

T47. Identification of Recombinant Antibodies for Functional and Structural Analysis of HIV-Host Complexes

Natalia Sevillano[1], Hai Ta[1], Jennifer Binning[1], Florencia La Greca[1], Judd Hultquist[2], Nathalie Caretta[1], Amber Smith[3], Xuefeng Ren[4], Bei Yang[4], Bhargavi Jayaraman[1], Shumin Yang[1], Yifan Cheng[3], Nevan J. Krogan[2], John Gross[1] and Charles S. Craik[1]

[1]Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, USA; [2]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA; [3]Department of Biochemistry and Biophysics, University of California, San Francisco, CA, USA; [4]Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA

Monoclonal antibodies (mAbs) are ubiquitous in biomedical research and medicine. Synthetic antibodies (recombinant antibodies, rAbs) can be created in the laboratory, completely eliminating animals from the antibody-production process. They have several advantages over the traditional hybridoma-based antibodies, including control over the state of the antigen that allows identification of antibodies to conformational states of the antigen, rapid identification of antibody binders allowing automation for high throughput production and ability to develop antibodies to highly toxic or non-immunogenic proteins. Besides their therapeutic potential, rAbs are also powerful research tools widely used in structural biology.

A fully human naïve Fab (fragment antigen-binding) phage-display library with a diversity of 4.1×10^{10} was constructed by the Craik laboratory using methods previously described. We have optimized the protocols for phage display panning for fast verification of binders and initial characterization of the epitope by semi-quantitative and competitive ELISAs without the purification of the Fabs. Using these optimized protocols, we have successfully generated Fabs that are useful for both functional and structural analysis against a wide range of protein targets including proteases, membrane proteins and protein complexes. We have also identified recombinant Fabs for HIV related targets: Vif-CBFβ-ELOB-ELOC complex (VCBC); the clathrin adaptor complex AP-2 and Nef; the endosomal sorting complexes required for transport I (ESCRT-I); and Rev-RNA Complexes.

For VCBC complex, we have identified six tight-binding Fabs, with binding constants in the nanomolar range, we have grouped these Fabs in three classes corresponding to independent epitopes. Two of these Fabs, 3C9 and 1D1 inhibit the ubiquitination of different APOBEC *in vitro* and *in vivo*. We are also using combinations of two or three of these Fabs for structural analysis of the complexes using negative stain and single particle electron cryo microscopy (cryoEM).

T48. VCBC-Specific Fabs Inhibit Ubiquitination and Degradation of A3 Proteins

Jennifer M. Binning[1], Amber Smith[4,5], Natalia Sevillano[1], Judd F. Hultquist[2,3], Nathalie Caretta Cartozo[1], Hai Ta[1], Nevan J. Krogan[2,3], Yifan Cheng[4,5], Charles S. Craik[1], John D. Gross[1]

[1]Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94158, USA; [2]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, California 94158, USA; [3]J. David Gladstone Institute, San Francisco, CA 94158; 4Howard Hughes Medical Institute, UCSF, San Francisco, CA 94158, USA; [4]Howard Hughes Medical Institute, UCSF, San Francisco, CA 94158, USA; [5]Department of Biochemistry and Biophysics, UCSF, San Francisco, CA 94158, USA

The severe pathogenesis associated with HIV is due in large part to its potent inhibition of host immune mechanisms. Among the known lentiviral accessory proteins, the virally encoded Vif protein plays an important role in counteracting the antiviral effects of host APOBEC3 (A3) innate immune proteins. Vif primarily antagonizes A3 by hijacking a cellular Cullin-RING ubiquitin ligase, resulting in the ubiqutination and subsequent targeting of A3 for proteasomal degradation. Vif-mediated inhibition of A3 is critical to lentiviral pathogenesis; therefore, targeting Vif is a promising strategy for the development of novel HIV inhibitors. To assess the potential of Vif as a therapeutic target, we generated Fabs against the HIV-1 Vif/EloB/EloC/CBF-β (VCBC) complex and tested their ability to inhibit A3 ubiquitination. Using our *in vitro* ubiquitination assay, we established that two Fabs, termed 3C9 and 1D1, successfully blocked VCBC-mediated A3 ubiquitination. Specifically, 3C9 inhibited ubiquitination of the A3F C-terminal domain (CTD), whereas 1D1 inhibited both A3F-CTD and A3G ubiquitination. An intracellularly expressed single-chain variant of 3C9 was able to inhibit Vif-mediated A3F degradation in celluo and, importantly, can restore A3F packaging into virions. Similarly, a single chain variant of 1D1 can restore levels of all tested A3 proteins in celluo, which is consistent with our in vitro results; however, the 1D1-restored A3 proteins were unable to be packaged into virions. We have thus identified VCBC-specific Fabs that bind the HIV-1 Vif complex with high affinity and specificity, and have the capacity to function as Vif-E3 ligase inhibitors. Our findings implicate Vif as a promising therapeutic target and experimentally establish the feasibility of generating inhibitors directed against the Vif-E3 complex.

T49. Cleavable Cross-linkers, Multistage Mass Spectrometry, and the Structural Characterization of APOBEC3-Vif-CRL5 Complexes

Robyn M Kaake[1], Seung Joong Kim[3], Ignacia Echeverria[3], Linda Chelico[4], John D Gross[3], Andrej Sali[3], Lan Huang[5], Nevan J Krogan[1,2]

[1]The J David Gladstone Institutes, Institute for Virology and Immunology, San Francisco, CA; [2]University of California San Francisco, Dept. of Cellular and Molecular Pharmacology, San Francisco, CA; [3]University of California San Francisco, Dept. of Pharmaceutical Chemistry, San Francisco, CA; [4]University of Saskatchewan, College of Medicine, Saskatoon, Canada; [5]University of California Irvine, Dept. of Physiology & Biophysics, Irvine, CA

APOBEC3 cytidine deaminases (e.g. A3G, A3F, and A3H-II) potently restrict HIV infection via lethal hypermutation of virus genomes. HIV-1 Vif co-opts a cellular transcription factor (CBFβ) and together acts as a non-endogenous substrate receptor for Cullin5-RING E3 ligases (CRL5). These hybrid Vif-CRL5 complexes target A3 restriction factors for ubiquitylation and subsequent proteasomal degradation. Elucidation of A3-Vif-CRL5 structures can highlight druggable protein interfaces and identify new targets for HIV therapies. Structural characterization of A3-Vif-CRL5 complexes by more traditional approaches (e.g. crystallography, NMR, EM) has proven difficult in part due to the transient nature of enzyme-substrate interactions, the stability and solubility of A3 proteins, and the inherent conformational heterogeneity and flexibility of CRL5 complexes. Structural cross-linking mass spectrometry (XL-MS) is an alternative methodology in which MS-identified cross-linked peptides are used to map protein-protein interaction interfaces and provide distance constraints for integrative structural determination. Importantly XL-MS: 1) stabilizes transient interactions; 2) accommodates conformational heterogeneity; 3) is compatible with most in solution conditions; 4) operates at relatively low concentrations and absolute amounts; and 5) has potential for in cell applications. Here, we use a specialized *in vitro* XL-MS strategy to interrogate the structure of A3-Vif-CRL5 complexes. Briefly, proximate lysine (K) residues of target complexes are cross-linked with MS cleavable cross-linker DSSO[1], proteins are separated by SDS-PAGE and in gel trypsin digested, and the resulting complex mixture of cross-linked and non-cross-linked peptides are analyzed by multistage MS (MS^n). Cross-linked peptides are unambiguously identified by traditional bottom-up MS search algorithms (Protein Prospector) and in house software[1,2]. In all we identify 4244 redundant and 157 unique K-K linkages, including 21 inter-molecular linkages between A3G-Vif and 39 between the other Vif-CRL5 components. Using integrative modeling to incorporate known structures and homology models with XL-MS data, we have generated a working structural model for the A3G-Vif-CRL5 complex. Our model corroborates published structures and genetic interface mapping and additionally captures previously undefined detail, thus demonstrating the feasibility of DSSO-MS^n methods for characterization of virus-host complexes.

1. Kao, A., et al., Mol Cell Proteomics. 2011, 10(1): M110.002212.
2. Kao, A., et al., Mol Cell Proteomics. 2012, 11(12): 1566-77.

T50. Global Landscape of Ubiquitylation and Phosphorylation Changes in Response to HIV-1 Infection Identifies a Novel Substrate of Vif

Jeffrey Johnson[1,2], David Crosby[1], Judd Hultquist[1], Erik Verschuren[3], Lara Manganaro[4], Billy Newton[1], Tasha Johnson[1], Tricia Lundrigan[1], Viviana Simon[4], Alan Frankel[1], and Nevan Krogan[1,2*]

[1]University of California San Francisco; [2]Gladstone Institutes; [3]Genentech, Inc.; [4]Icahn School of Medicine at Mount Sinai; [*]Corresponding author

The human immunodeficiency virus type-1 (HIV-1) extensively remodels its host environment in order to create conditions optimal for viral replication and immune evasion. The virus has evolved to hijack the ubiquitin proteasome system to promote the degradation of antiviral factors such as APOBEC3G and tetherin. HIV-1 also modulates myriad signaling pathways affecting the cell cycle, transcriptional regulation, and innate immune processes. Identifying proteins whose post-translational modification state is altered during the course of infection helps to gain a comprehensive understanding of the pathways and processes modulated by the virus.

We applied quantitative proteomics to globally measure changes in host protein ubiquitylation and phosphorylation in response to HIV-1 infection. Additionally, mutant viruses were profiled to map the activity of post-translational modification events to specific accessory genes. This approach was able to identify known substrates of HIV-mediated ubiquitination and degradation, including APOBEC3C, tetherin, and CD4, as well as known phosphorylation events that are the hallmark of cell cycle arrest induced by HIV-1. Additionally, hundreds of novel ubiquitylation and phosphorylation events were observed to change significantly in response to HIV infection.

Among the novel ubiquitlyation events that were identified, we found that the protein phosphatase 2A (PP2A) B56 regulatory subunit family was targeted for ubiquitylation and degradation during HIV-1 infection. This activity was mapped to the HIV-1 accessory gene Vif. Co-transfection, ubiquitin immunoprecipitation, and Western blotting experiments validated these findings. Experiments testing a panel of Vifs derived from clinical HIV-1 isolates found differences between lentiviral Vif alleles in their ability to degrade B56 that were not correlated with other defined Vif activities. Phosphoproteome analysis of cells infected with wild-type and Vif-deficient virus identified a putative substrate of PP2A B56 that is required for proper innate immune sensing of HIV-1 and is under strong positive selection. The Vif gene has been previously shown to suppress innate immune sensing pathways in monocyte-derived macrophages and dendritic cells. Our systems biology approach led us to a specific hypothesis addressing how this suppression is actually accomplished, and current experiments seek to test this hypothesis.

T51. Rerouting Resistance: Escaping Restriction Using Alternative Cellular Pathways

Aya Khwaja, Meytal Galilee, Ailie Marx, and Akram Alian

Faculty of Biology, Technion – Israel Institute of Technology, Haifa 320003, Israel

The AIDS pandemic continues to devastate tens of millions of people around the world as the HIV-1 virus continues to stubbornly evade intrinsic host and therapeutically administered opposition. Therapeutic strategies are shifting from traditional approaches of targeting viral proteins, which continues to mutate and gain resistance, to an interruption of key host-virus interactions. We hypothesize, however, that redundancies in cellular mechanisms may afford the virus with an important survival advantage by exploiting alternative cellular machineries when the default pathway is blocked by antiviral therapeutics or cellular restriction (1,2). HIV-1 has indeed been shown to exploit alternative cellular routes in escaping drugs targeting vital cellular cofactors such as escaping Maraviroc inhibition of CCR5 by utilizing the alternative CXCR4 coreceptor, and adapting to PF74 targeting of viral capsid by altering the pattern of cellular cofactor utilization during nuclear entry.

We propose that virus flexibility in rerouting within essential cellular pathways will lead to a new type of stubborn resistance, 'rerouting resistance,' and that truly effective anti-AIDS drugs can only be designed when a more holistic understanding of host–virus interactome, including potential rerouting landscapes, is adopted. We also propose an approach in which cross-family differences can aid highlighting resistance-mechanisms accessible to challenged HIV-1 by mutational adaptation. In this approach, we suggest exploring how related viruses coevolved in their natural environment to uncover hidden alternative routes and replication patterns potentially accessible to emergent HIV-1 strains.

To illustrate the viability of the 'rerouting' concept we will discuss the molecular mechanisms by which mutant viral proteins may regain stability and folding. Fitness viral proteins may employ either (or both) an *intrinsic* mechanism, through the accumulation of *coevolved mutations* that induce compensatory conformational changes, or by an *extrinsic* mode via the interaction with molecular chaperoning proteins promoting the acquisition of the functional folded state. We examined the *intrinsic mechanism* prospect and, in addition to providing a novel crystal structure of FIV capsid C-terminal domain, we reveal an alternative but correlated pattern of coevolved substitutions, which when otherwise uncoupled and individually substituted into HIV-1 capsid impairs virus infectivity. Flexibility of lentiviral Vif protein in exploiting various cofactors presents a viable example on *extrinsic mechanisms* of protein evolution. While lentiviral Vif hijacks the E3-ubiquitin complex in evading APOBEC3 cellular-restriction, analysis suggests flexibility in exploiting a diversity of cellular cofactors providing alternative HIV-1 rescue mechanisms.

Pathway targeting is apparently not unique to HIV–host interactions and not even an innovation of parasitic pathogens, but rather a common biologic principle. Therefore, embracing the 'rerouting' concept not only has obvious applicability to numerous pathways utilized by HIV-1 replication, and virology in general, but also will open up new horizons for exploring pathway-specific alterations in normal and diseased cellular states, including cancer.

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2. Marx, A., and Alian, A. (2015) Rerouting Resistance: Escaping Restriction Using Alternative Cellular Pathways. *Trends Microbiol* **23**, 595-597

T52. HIV-1 Interaction with CypA Regulates Use of FG-Nucleoporins for Nuclear Entry

Guangai Xue[1], Shih Lin Goh[2], Hyun Jae Yu[1], Anna T. Gres[3], KyeongEun Lee[1], Stefan G. Sarafianos[3], Jeremy Luban[2], and Vineet N. KewalRamani[1]

[1]Basic Research Laboratory, National Cancer Institute, Frederick, MD; [2]Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA; [3]Bond Life Sciences Center, MMI, Biochemistry, University of Missouri, Columbia, MO

We performed a small-interfering RNA screen targeting all known human nucleoporins (Nups) to assess effects on HIV-1 infection. We identified Nup35 and POM121 as potential HIV-1 cofactors not previously detected in prior screens. HIV-1 reliance on Nup35 or POM121 was linked to the viral capsid protein (CA). Depletion of either protein impaired infection by wild-type (WT) HIV-1 but not virus bearing N74D or P90A mutant CA. Initial experiments focused on Nup35. CRISPR/Cas9 knockout experiments confirmed a contribution by Nup35 to HIV-1 infection, and quantitative PCR detection of reverse transcription products in Nup35 knockdown cells indicated a block to viral nuclear entry. Nup35 and POM121 are phenylalanine-glycine Nups (FG-Nups). Approximately one-third of the Nups are FG-Nups, including HIV-1 cofactors Nup153 and Nup358. FG-Nups contain multiple FG-dipeptides, maintain the nuclear diffusion barrier, and provide docking sites for nuclear transport receptors (NTRs). It was previously demonstrated that the N74 pocket of CA interacts with FG-motifs from CPSF6 and Nup153. We examined the contribution of Nup35 FG-dipeptides in supporting HIV-1 infection. While ectopically expressed WT Nup35 restored HIV-1 infection in Nup35 knockdown cells, Nup35 with a singular FG disruption was incapable of rescuing infection in the cells depleted of endogenous protein. Notably, HIV-1 interaction with cyclophilin A (CypA) regulated dependence on the FG-Nups. Disruption of the interaction between CA and CypA either by cyclosporine A (CsA) treatment or CypA knockdown restored WT HIV-1 infectivity in Nup35 knockdown cells. Moreover, using a microscopy assay for HIV-1 nuclear entry, we observed evidence of CA accumulation in the nucleus of Nup35, POM121, and Nup153 knockdown cells only after treatment with CsA. We hypothesize that CypA use by HIV-1 alters its CA conformation and exposes the N74 containing pocket to mediate interactions with different FG-Nups present in the nuclear pore complex (NPC). We propose that HIV-1 exploits successive FG interactions between its core, likely containing hundreds of CA molecules, and FG-Nups to achieve transfer through the NPC. In this model, the HIV-1 core directly functions as a NTR capable of multiple low affinity interactions with FG-dipeptides.

T53. HIV-1 Nuclear Trafficking is Altered by Cytoplasmic CPSF6 Expression in a Capsid-Dependent Manner

Zhou Zhong[1], Callen Wallace[2], Christopher Kline[3], Simon C. Watkins[2], and Zandrea Ambrose[1,3]

[1]Molecular Virology and Microbiology Program, [2]Department of Cell Biology, and [3]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

Trafficking of HIV-1 particles to the nucleus is poorly understood. Previous studies showed that depletion of transportin-3 (TNPO3) or expression of a truncated form of a TNPO3 nuclear importin, cleavage polyadenylation specific factor 6 (CPSF6-358), inhibits nuclear entry of HIV-1 DNA. CPSF6 binds to both TNPO3 and HIV-1 capsid and is expressed mainly in the cell nucleus. We hypothesized that nuclear or peri-nuclear localization of CPSF6 is required for proper viral trafficking and relocalization of CPSF6 to the cell periphery alters trafficking of viral DNA. Using fluorescently labeled integrase packaged into HIV-1 particles, we performed live-cell swept field confocal imaging, total internal reflection fluorescence microscopy, and fixed cell confocal imaging of infected HeLa cells expressing CPSF6-GFP or CPSF6-358-GFP. We compared WT HIV-1 with a capsid mutant, N74D, which does not bind to CPSF6. Three-dimensional reconstruction of infected cells was performed and tracking data of HIV-1 particles in cells was analyzed. We observed the formation of higher order complexes of cytoplasmic CPSF6 associated with microtubules after WT HIV-1 infection. Initial co-localization of fluorescent integrase with CPSF6 higher-order complexes was observed, followed by the viral particle movement away on microtubules. However, aggregation of CPSF6 did not occur when cells were infected with the capsid mutant or treated with a small molecule inhibitor, PF74, which prevents capsid from binding to CPSF6. While WT HIV-1 particles had relatively linear tracks in cells expressing nuclear CPSF6, relocalization of CPSF6 to the cell periphery altered the cytoplasmic trafficking of viral particles. Our data suggests that premature access of CPSF6 by HIV-1 capsid leads to the formation of higher order complexes and alteration of cytoplasmic trafficking towards the nucleus.

T54. HIV-1 Capsid Protein Modulates the Activity of Preintegration Complexes

Muthukumar Balasubramaniam[1], Amma Addai[1], Jing Zhou[2], Jui Pandhare[1], Christopher Aiken[2], and Chandravanu Dash[1]

[1]Center for AIDS Health Disparities Research, Meharry Medical College, Nashville, TN; [2]Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN

HIV-1 capsid (CA) is a multifunctional viral protein and an attractive target for novel therapeutics. CA forms the conical viral capsid, which surrounds the viral ribonucleoprotein complex and facilitates early post entry steps in infection. While it is well established that the CA is involved in reverse transcription and nuclear entry, genetic studies provide indirect evidence that CA also controls HIV-1 integration. Recent studies also show that HIV-1 CA co-stains with integrase (IN) in the cytoplasm, at/near the nuclear membrane and even inside the nucleus of infected cells. Moreover, a recent report indicated that CA can enter the nucleus in association with HIV-1 preintegration complexes. However, data supporting a direct and functional link between HIV-1 CA and integration are lacking. To probe a role of CA in integration, we employed a biochemical approach that measures integration activity and composition of HIV-1 pre-integration complexes (PICs) isolated from the cytoplasm (Cy-PICs) and nucleus (Nu-PICs) of acutely infected cells. We used the antiviral compound PF74 that binds to CA and known to alter capsid stability in vitro. In a T cell line, our data show that 2 μM PF74 inhibits infection by 90% activity but has minimal effect on reverse transcription, indicating that the compound alters subsequent steps of HIV-1 infection. Using Alu PCR-based assays of HIV 1 integration in target cells, we observed that infection in the presence of 2 μM PF74 resulted in decreased levels of integrated HIV-1 DNA, likely owing to inhibitory effects on both nuclear entry and integration. We hypothesized that PF74 inhibits the activity of Cy-PICs by promoting capsid retention. Surprisingly, the PICs exhibited markedly increased elevated levels of integration activity despite normal levels of viral DNA. The PF74-induced increase in integration activity was not exhibited by PICs isolated from cells inoculated with a PF74-resistant HIV-1 mutant (5Mut), demonstrating that the effect was a consequence of PF74 acting on the viral capsid. We propose that PF74 enhances PIC activity by partial destabilization of the core, thus uncloaking the PICs for integration. Alternatively, PF74 may stabilize the viral capsid in target cells, thereby protecting the reverse transcribed viral genome and leading to more efficient integration. To distinguish between these hypotheses, we are currently assaying the specific integration activity of PICs of specific CA mutants with hyperstable and unstable capsid. Activity measurements of PICs of the E45A mutant with a hyperstable capsid show significantly reduced integration activity relative to the wild type PICs. Interestingly, PIC activity of the double mutant E45A/R132T with a revertant phenotype exhibited elevated levels of viral DNA integration compared to E45A PICs. These data suggest that increasing capsid stability renders reduced integration activity of PICs. Collectively, these studies strongly suggest a direct and functional role of HIV-1 CA in viral DNA integration.

T55. Modeling Ligand Binding to an Allosteric Site in the Catalytic Core Domain of HIV-1 Integrase Using Absolute and Relative Free Energy Methods

Nanjie Deng,[1] Stefano Forli,[2] Joseph Bauman,[3] James Fuchs,[4] Mamuka Kvaratskhelia,[4] Alan Engelman,[5] Eddy Arnold,[3] Art Olson,[2] and Ronald Levy[1]

[1]Center for Biophysics and Computational Biology, Temple University, Philadelphia, PA; [2]The Scripps Research Institute, La Jolla, CA; [3]Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ; [4]College of Pharmacy, Ohio State University, Columbus, OH; [5]Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

HIV-1 integrase (IN) is essential for virus replication and represents an important therapeutic target. Recently discovered quinoline-based allosteric IN inhibitors (ALLINIs), which compete with LEDGF/p75 for binding to IN, potently impair HIV-1 replication and have been evaluated in clinical trials. ALLINIs inhibit HIV-1 by inducing aberrant IN multimerization, which impairs the encapsidation of the viral ribonucleoprotein complex within the viral capsid during virus maturation. Because ALLINIs have a relatively low genetic barrier for the evolution of resistance phenotypes, there is a need for discovery of second generation inhibitors. Using crystallographic fragment screening against the HIV-1 IN catalytic core domain (CCD) followed by a fragment expansion approach, we have identified thiophenecarboxylic acid (TPCA)-derivatives that bind at the CCD-CCD dimer interface at the principal LEDGF/p75 binding pocket.

We have performed binding free energy simulations on three TPCA derivatives bound at the LEDGF-binding pocket of IN. The goal of the computational study is to predict the binding affinities and binding modes of these compounds in solution and to understand the energetics and the effects of ALLINI resistant mutations H171T and A128T on the binding of the fragments. The calculations predict that compounds **5** and **8** bind significantly more strongly compared with compound **1**, and that the predicted binding dissociation constants (K_d) of **1**, **5** and **8** show good correlation with experimentally measured IC_{50}s. The calculations also show that the H171T mutation only slightly modifies the binding affinities of the three compounds**,** by less than 2 fold. In contrast, H171T is reported to weaken the binding of quinoline based ALLINI BI-D by ~65 fold. The lack of susceptibility of the TPCA compounds to H171T is believed to be related to the absence of a tert-butoxy moiety in these compounds, as the tert-butoxy functional group in BI-D forms strong intermolecular H-bonds with the side chain of H171. Similar to prior reports of quinoline-based compounds, the A128T mutation was estimated to have a very small effect on the binding affinities of the fragments studied here.

We have also carried out large scale relative binding free energy calculations using a novel thermodynamic cycle that we call the Common Intermediate State Method to predict a priori the binding affinities of a series of ligands designed from the recently reported pyridine-based ALLINI KF115. The method utilizes common intermediate states to avoid difficult alchemical transformations like ring expansion and breaking in the calculation of relative free energy. Here we focused our effort on a small library of KF115 derivatives with varying R1 groups, and have identified several of them that show better or comparable binding affinities compared with the parental inhibitor.

T56. HIV-1 Integrase Strand Transfer Inhibitors that Reduce Susceptibility to Drug Resistant Mutant Integrases

Xue Zhi Zhao,[1] Hannah Peters,[1] Steven J. Smith,[2] Daniel P. Maskell,[4] Mathieu Metifiot,[3] Valerie E. Pye,[4] Katherine Fesen,[3] Christophe Marchand,[3] Yves Pommier,[3] Peter Cherepanov,[4,5] Stephen H. Hughes,[3] and Terrence R. Burke, Jr.[1]

[1]Chemical Biology Laboratory and [2]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD 21702; [3]Developmental Therapeutics Branch and Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; [4]Clare Hall Laboratories, The Francis Crick Institute, Blanche Lane, South Mimms, EN6 3LD, UK; [5]Imperial College London, St-Mary's Campus, Norfolk Place, London, W2 1PG, UK

HIV integrase (IN) strand transfer inhibitors (INSTIs) are among the newest anti-AIDS drugs. There are currently three FDA-approved HIV-1 INSTIs: Merck's Raltegravir (RAL) (October 2007); Gileads' Elvitegravir (EVG) (August 2012) and GlaxoSmithKlines' Dolutegravir (DTG) (August 2013). However, the emergence of drug-resistant IN mutants emphasizes the need to develop additional agents that have improved efficacies against the existent resistant mutants. We developed non-cytotoxic naphthyridine-containing INSTIs that retain low nanomolar IC_{50} values against HIV-1 variants harboring all of the major INSTI-resistant mutations. We found by analyzing crystal structures of inhibitors bound to the highly homologous prototype foamy virus (PFV) integrase, that the most successful inhibitors show striking mimicry of the bound viral DNA prior to 3'-processing and the bound host DNA prior to strand transfer. Using this concept of "bi-substrate mimicry" we developed a new broadly effective inhibitor that not only mimics aspects of both the bound target and viral DNA, but also more completely fills the space they would normally occupy. Maximizing shape complementarity and recapitulating structural components encompassing both of the IN DNA substrates could serve as a guiding principle for the development of new INSTIs.

T57. Selectivity for Strand-Transfer Over 3'-Processing and Susceptibility to Clinical Resistance of HIV-1 Integrase Inhibitors Are Both Driven by Key Enzyme-DNA Interactions in the Active Site

Mathieu Métifiot[1], Barry C. Johnson[2], Evgeny Kiselev[1], Laura Marler[1], Xue Zhi Zhao[3], Terrence R. Burke Jr[3], Christophe Marchand[1], Stephen H. Hughes[2] and Yves Pommier[1]

[1]Developmental Therapeutics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; [2]HIV Drug Resistance Program, Frederick National Laboratory for Cancer Research, Center for Cancer Research, National Cancer Institute-Frederick, National Institutes of Health, Frederick, MD 21702; [3]Chemical Biology Laboratory, Frederick National Laboratory for Cancer Research, Center for Cancer Research, National Cancer Institute-Frederick, National Institutes of Health, Frederick, MD 21702

Integrase strand transfer inhibitors (INSTIs) are highly effective against HIV infections. Co-crystal structures of the prototype foamy virus intasome have shown that all three FDA-approved drugs, raltegravir, elvitegravir and dolutegravir, act as interfacial inhibitors during the strand transfer integration step. However, these structures give only a partial sense for the limited inhibition of the 3´-processing reaction by INSTIs and how INSTIs can be modified to overcome drug resistance, notably against the G140S-Q148H double mutation. Based on biochemical experiments with modified oligonucleotides, we demonstrate that both the viral DNA +1 and -1 bases, which flank the 3´-processing site, play a critical role for 3´-processing efficiency and inhibition by raltegravir and dolutegravir. In addition, the G140S-Q148H (SH) mutant integrase, which has a reduced 3´-processing activity becomes more active and more resistant to inhibition of 3´-processing by raltegravir and dolutegravir in the absence of the -1 and +1 bases. Molecular modeling of HIV-1 integrase, together with biochemical data, indicate that the conserved residue Q146 in the flexible loop of HIV-1 integrase is critical for productive viral DNA binding through specific contacts with the virus DNA ends in the 3´-processing and strand transfer reactions. The potency of integrase inhibitors against 3´-processing and their ability to overcome resistance is discussed.

T58. Using Fragment-Based Analysis of Virtual Screenings to Characterize Binding Sites

Richard K. Belew[1], Stefano Forli[2], David Goodsell[2], T. J. O'Donnell[3], and Arthur Olson[2]

[1]Univ. California – San Diego (rbelew@ucsd.edu); [2]The Scripps Research Institute; [3]gNova

In order to help analyze the data from our large FightAIDS@home virtual screens we have developed ADChemCast, a representation of a protein structure in terms of receptor-ligand interactions found to be shared across AutoDock docking of many ligands in a virtual library. Details of the specific interactions between residue and ligand atoms underlying the docking are localized to a virtual fragmentation of the ligands using RECAP. While neither the residue-ligand atom interaction features nor the fragmentation influence AutoDock's docking energy calculation, they do support post hoc analysis of patterns of receptor interaction with fine-grained features of ligand components.

We have used ADChemCast as the basis for classification of proteins included in the Database of Useful Decoys -- Enhanced (DUD-E) experimental set, and demonstrated strong performance against competitive results. We use interpretable machine learning methods to provide insight into just how active compounds can be described in terms of residue-ligand atom interaction features and fragments. In addition to exploiting dominant hydrogen bond interactions, identifying consistent patterns in ligand's atoms' van der Waals (vdW) interactions across the receptor surfaces has proven critical. In the case of the HIV-1 reverse transcriptase enzyme (part of DUD-E), we also demonstrate relevance of these results by showing the overlap of these same features with approved inhibitors.

We also describe current efforts to extend these methods to investigate quinoline-based allosteric HIV-1 integrase (IN) inhibitors (ALLINIs) and related pyridine-based multimeric IN inhibitors (MINIs). Known potent inhibitors are used as queries against ADChemCast indices of the virtual libraries to retrieve library compounds that share similar fragments and similar binding features to suggest new compounds for biological assay.

T59. Structure-Selective Endonuclease MUS81/EME1 Downregulation and G2 Cell Cycle Arrest are Independent Functions of Vpr

Xiaohong Zhou, Maria DeLucia, and Jinwoo Ahn

Department of Structural Biology and Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA

HIV-1 Vpr regulates multiple activities in virus life cycles, including nuclear import of pre-integration, induction of cell cycles arrest, stimulation of virus transcription and downregulation of host factors. Recently, Vpr was reported to downregulate structure-selective endonuclease MUS81. Evolutionarily conserved MUS81 forms a complex with EME1, and further associates with another endonuclease SLX4/SLX1 to form a four-subunit complex of MUS81/EME1/SLX4/SLX1, coordinating distinctive biochemical activities of both endonucleases in DNA repair. Vpr was previously reported to bind SLX4 to mediate downregulation of MUS81, which appears to link to G2 arrest in cycling cells. However, the detail mechanism underlying the MUS81 downregulation is not clear yet. Here we show that the MUS81/EME1 is downregulated by ubiquitin-proteasome pathway, via Vpr hijacking the host CRL4-DCAF1 E3 ubiquitin ligase. Multiple Vpr variants from HIV-1 and SIV downregulate both MUS81 and its cofactor EME1. We also find a C-terminally truncated Vpr mutant and single residue mutants, which lost G2 arrest activity, are still capable of downregulating MUS81 and EME1, suggesting that G2 arrest and MUS81/EME1 downregulation are independent functions of Vpr. Interestingly, neither the interaction of MUS81/EME1 with DCAF1/Vpr nor their downregulation is dependent on SLX4/SLX1. Therefore, information from this study underlines how Vpr regulates host factors downregulation and may be help in developing anti-HIV-1 strategies.

T60. To Repair or Not Repair: The X-ray Structure of the DDB1-DCAF1-Vpr-UNG2 Complex

Ying Wu[1], Xiaohong Zhou[1], Christopher O. Barnes[2], Maria DeLucia[1], Aina E. Cohen[3], Angela M. Gronenborn[1], Jinwoo Ahn[1], and Guillermo Calero[1]*

*[1]Department of Structural Biology and Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA; [2]Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA; [3]Stanford Synchrotron Radiation Lightsource, Menlo Park, CA 94025, USA; *Correspondence: jia12@pitt.edu (J.A.), guc9@pitt.edu (G.C.)*

Uracil-DNA glycosylase (UNG2) is crucial in preserving genome fidelity and stability by repairing uracil-DNA lesions during replication. The role of UNG2 during AIDS pathogenesis has remained controversial over several decades. The recruitment of UNG2 into virus particles by HIV-1 accessory protein R (Vpr) is important for efficient viral infection of macrophages. In addition, Vpr has been shown to promote UNG2 degradation through the host ubiquitin proteasome pathway via interacting with DDB1- and CUL4A-associated factor 1 (DCAF1), a cellular substrate receptor of the Cullin4-RING E3 ubiquitin ligase machinary (CRL4). Here, we present the crystal structure of HIV-1 Vpr and human UNG2 in complex with the CRL4 receptor-adaptor proteins DDB1-DCAF1. The structure reveals the elegant mechanism employed by Vpr to “mimic” damaged-DNA, when binding to UNG2, thereby inhibiting its catalysis activity. Moreover, Vpr also steers UNG2 towards destruction by loading it onto CRL4 ubiquitin ligase machinary.

T61. Human T-Cell Leukemia Virus Type 1 Proviral Load and Genome Structure in Chronically Infected T-Cell Lines

Morgan E Meissner[1,2], LeAnn Oseth[5], Jessica L Martin[1,3], Luiza Mendonca[1], Wei Zhang[1,4], Louis M Mansky[1,2,3]

[1]University of Minnesota, Institute for Molecular Virology, Minneapolis, MN, [2]University of Minnesota, Molecular, Cellular, Developmental Biology, and Genetics Graduate Program, Minneapolis, MN, [3]University of Minnesota, Pharmacology Graduate Program, Minneapolis, MN, [4] University of Minnesota, Characterization Facility, Minneapolis, MN, [5]University of Minnesota, Cytogenomics Core, Minneapolis, MN

Human T-cell leukemia virus type-1 (HTLV-1) infects about 15 million people worldwide and results in an incurable leukemia in about 5% of individuals infected. Progress in the study of the mechanisms of viral replication and virus particle structure have been limited given the difficulty in propagating the virus in cell culture. MT-2 cells have been extensively used as a source of infectious HTLV-1 particles. However, a limitation is that these cells have multiple proviruses, some of which contain genome perturbations that could produce non-infectious particles with altered virus particle morphologies. To address this potential limitation in the use of MT-2 cells as a homogenous source of infectious HTLV-1 particles, a panel of cell lines were analyzed for their proviral content by using fluorescence in-situ hybridization. Analysis of this panel identified the SP cell line as a promising cell line that contained only for 4 proviruses, compared to other cell lines that harbored as many as 21 proviruses. Proviral integration sites in SP cells were determined via Splinkerette PCR analysis. Nucleotide sequencing was used to confirm the proviral structure, particularly for intact Gag and protease coding sequences. Cryo-electron microscopy of virus particles produced from SP cells provides evidence of HTLV-1 particles with variable particle morphologies. Ongoing studies are directed at investigating relative virus infectivity of particles compared to that from MT-2 cells. Taken together, our studies indicate that the SP cell line represents a promising cell line for the production of HTLV-1 particles produced from intact proviral sequences. Such a reagent provides a standardized cell line for use in experiments investigating authentic virus particle morphology.

M.M. is supported by the Institute for Molecular Virology Training Program (NIH T32 AI083196), University of Minnesota-Twin Cities.

T62. Structural Basis of an Evolved Cre Recombinase that Excises HIV DNA

Gretchen Meinke[1], Janet Karpinski[2], Frank Buchholz[2] and Andrew Bohm[1]

[1]Department of Developmental, Molecular and Chemical Biology, Tufts University School of Medicine, Boston, MA, 02111, USA; [2]Medical Systems Biology, UCC, Medical Faculty Carl Gustav Carus, TU Dresden, Germany

We are pursuing a genome editing approach to excise the HIV provirus from infected cells using engineered versions of the Cre recombinase. These engineered recombinases, termed Tre and Brec1, recognize unique 34 bp (base-pair) sequences present in the HIV long terminal repeats (LTRs). Upon LTR binding, these recombinases can then excise the provirus. Like Cre, they do not need additional co-factors, and they act with single-nucleotide precision and do not rely on error-prone repair mechanisms for chromosomal ligation after removal of the provirus DNA. Thus, a Cre-based therapy may prove safer than nuclease-based therapies such as CRISPR-Cas9. This novel approach does not require that the targeted cells be actively producing the virus, and therefore it can, in principle, act on latently-infected cells.

For recombination to occur, a synaptic tetrameric complex comprised of four molecules of Cre interacting with two loxP sites is formed. LoxP consists of two perfect, inverted 13 bp repeats flanking an 8 bp spacer. As proof-of-principle, using directed evolution techniques, the Buchholz lab engineered a recombinase that recognizes a 34 bp sequence found within HIV-1 subtype A (termed loxLTR). This recombinase termed Tre, contains 19 mutations relative to Cre. The loxLTR site differs in 17 of the 34 positions relative to loxP, and is asymmetric. To understand how the engineered Tre interacts with loxLTR, we have solved the X-ray structure of Tre/loxLTR complex using a catalytically inactive point mutant (Y324F). We have analyzed the protein-DNA interactions and compared them to wt Cre/loxP structures. In addition, we can analyze how Tre interacts with non-identical sequences. For example, Arg 94 (Tre) forms a hydrogen bond to the N7 atom of Ade in one half-site, whereas at the other half-site, steric collisions with a Thy would occur, so the Arg 94 side-chain must move and it interacts instead with the phosphate backbone.

The Buchholz lab has recently engineered a second generation recombinase termed Brec1 that recognizes a 34 bp sequence that occurs in > 80 % of all HIV LTRs, and, hence may prove to be a more useful anti-HIV therapeutic than Tre. When delivered via a lentiviral vector, Brec1 was shown to efficiently excise integrated provirus from patient derived cells. No deleterious effects to mice were observed even when Brec1 was constitutively overexpressed. Crystal structures of Tre, Brec1 and related recombinases will allow us to better understand how to alter specificity of these enzymes and enhance their activity and utility.

T63. DNA-PK Inhibition Potently Represses HIV Transcription and Replication

Geetaram Sahu[1], Kalamo Farley[1], Gary Simon[1] and Mudit Tyagi[1, 2]

[1]Division of Infectious Diseases, Department of Medicine, George Washington University, Washington, DC 20037; [2]Department of Microbiology, Immunology and Tropical Medicine, George Washington University, Washington, DC 20037

DNA-dependent protein kinase (DNA-PK), a nuclear protein kinase that specifically requires association with DNA for its kinase activity, plays important roles in the regulation of different DNA transactions, including transcription, replication and DNA repair, as well as in the maintenance of telomeres. We reported DNA-PK facilitated HIV transcription by interacting with and phosphorylating the carboxyl terminal domain (CTD) of the RNA polymerase II (RNAP II) complex recruited to HIV LTR. In our current study, DNA-PK inhibition via highly specific small molecule inhibitors replicated the shRNA-mediated abrogation of both HIV transcription and replication in HIV infected myeloid and lymphoid cell lines, the main cell types targeted by HIV. These inhibitors also impaired HIV replication in primary CD4+ T-cells. Pre-treatment of the HIV-infected cell lines with the DNA-PK inhibitors also resulted in severe impairment of the phosphorylation of the serine 2 and serine 5 of the RNAP II CTD. Chromatin immunoprecipitation (ChIP) analysis showed that DNA-PK inhibition led to the establishment of transcriptionally repressive heterochromatin structures at the HIV LTR. These findings confirm the important role of DNA-PK in HIV transcription and replication. Moreover, we found that DNA-PK inhibitors successfully limit the reactivation of latent HIV proviruses in patients' PBMCs. This observation presents a strong evidence for the inclusion of transcription inhibitors, such as DNA-PK inhibitors as supplements to HAART regimens, in order to further enhance restriction of HIV replication, besides limiting transcription from proviruses and resultant deleterious effects from viral proteins.

T64. Identification of Smac Mimetics as a Novel Class of HIV-1 Latency Reversing Agents

Lars Pache[1], Miriam S. Dutra[1], Adam M. Spivak[2], John M. Marlett[3], Jeffrey P. Murry[3§], Young Hwang[4], Ana M. Maestre[5], Lara Manganaro[5], Mitchell Vamos[1], Peter Teriete[1], Laura J. Martins[2], Renate König[1,6], Viviana Simon[5], Alberto Bosque[2], Ana Fernandez-Sesma[5], Nicholas D. P. Cosford[1], Frederic D. Bushman[4], John A. T. Young[3#], Vicente Planelles[2] and Sumit K. Chanda[1]

[1]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037; [2]University of Utah School of Medicine, Salt Lake City, UT 84112; [3]The Salk Institute for Biological Studies, La Jolla, CA 92037; [4]Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104; [5]Icahn School of Medicine at Mount Sinai, New York, NY 10029; [6]Paul-Ehrlich-Institut, 63225 Langen, Germany; [§]Present address: Gilead Sciences, Foster City, CA 94404; [#] Present address: F. Hoffmann-La Roche Ltd, 4070 Basel, Switzerland

Replication of HIV-1 is highly dependent on components of the host cell machinery. To identify constitutively expressed factors that limit HIV-1 replication we selected a set of candidate genes that have the potential to impede viral replication based on a meta-analysis of multiple genome-wide screening datasets. Employing a targeted RNAi screen we identified 139 factors that interfere with early stages of HIV-1 replication. A subset of these factors were subjected to additional validation and mapped to specific stages of the viral life cycle.

One of these factors, BIRC2, limits HIV-1 transcription through an NF-κB-dependent mechanism. BIRC2 is a negative regulator of non-canonical NF-κB signaling, suggesting that this pathway activates HIV-1 transcription. Consistently, activation of non-canonical NF-κB signaling through BIRC2 depletion enhances HIV-1 replication. Interestingly, treatment of latently infected Jurkat (JLat) cells with small-molecule BIRC2 antagonists known as Smac mimetics led to a reactivation of the virus in this latency model. Moreover, Smac mimetics acted synergistically with HDAC inhibitors to reverse latency in JLat cell lines. We further confirmed the latency-reversing activity of these compounds in the more physiological context of resting $CD4^+$ T cells isolated from aviremic HIV-infected patients. We have found that Smac mimetic treatment, in combination with the HDAC inhibitor panobinostat, resulted in synergistic activation of the latent reservoir in this *ex vivo* system.

While previous studies have demonstrated that canonical NF-κB signaling is utilized by HIV-1 to increase viral replication, the role of the non-canonical NF-κB pathway has been more elusive. Taken together, our findings suggest that non-canonical NF-κB signaling increases HIV transcription. Moreover, activating the non-canonical NF-κB pathway by targeting BIRC2 with Smac mimetics appears as a promising new therapeutic strategy to reactivate latent HIV-1 while limiting toxicity risks associated with systemic activation of canonical NF-κB signaling.

T65. Visualization of Transcriptional Activation from HIV Integration Sites in Latently Infected Cells

Obiaara Ukah[1,2], Maritza Puray-Chavez[1,2], Juan Ji[1], Stefan G Sarafianos[1,2,3,4]

[1]University of Missouri, C.S. Bond Life Sciences Center, Columbia, MO, [2]University of Missouri, Molecular Microbiology & Immunology, Columbia, MO, [3]University of Missouri, Veterinary Pathobiology, Columbia, MO, [4]University of Missouri, Biochemistry, Columbia, MO

The HIV latent reservoir (LR) in patients is a major obstacle preventing successful eradication of viral infection. Methods that are currently available to detect the frequency of integrated viral DNA (vDNA) in latently infected cells fail to detect or accurately identify all transcriptionally silent replication competent proviruses. As a result, the size of the HIV LR may be overestimated if performing PCR amplification of vDNA, because this method can also detect replication incompetent viruses. Alternatively, the LR size may be underestimated if viral outgrowth assays are performed, because they may fail to reactivate all replication competent proviruses. Therefore, a sensitive method for direct visualization of integrated vDNA and the viral RNA (vRNA) transcribed from these integration sites would help to accurately estimate the size of the LR in patients.

Our lab has developed an assay to simultaneously detect single copies of vDNA and single copies of vRNA transcribed from integrated vDNA by using a known branched DNA *in situ* hybridization method on fixed latently infected HIV cells. To validate this assay, we used JLat cells, a model of HIV latency that produces vRNA and GFP upon stimulation with latency reversing agents (LRAs) including phorbol 12-myristate 13-acetate (PMA) and Ionomycin, TNF-α, or romidepsin, which allowed visualization of spliced and unspliced vRNA that could be monitored in a time-dependent manner. The development of this sensitive methodology for visualization of single presumptively integrated vDNA copies and furthermore, visualizing reactivation of transcriptionally silent proviruses *via* detection of single copies of spliced or unspliced vRNA transcribed from said integration sites, will help define the size of the LR and can ultimately be applied as a screening tool for future LRAs.

T66. Heterogenic Transcription Start Sites of HIV-1 and Their Influence on RNA Fates

Siarhei Kharytonchyk[1], Philip Smaldino[1], Sarah Monti[2], Michael F. Summers[2], and Alice Telesnitsky[1]

[1]Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor MI 48109-0620; [2]Howard Hughes Medical Institute and Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

Three guanine bases (G454, G455, and G456) reside at the beginning of the R region in HIV-1 DNA, and controversial data exists on which of these three “G”s serves as the transcription start site (TSS) during HIV-1 transcription initiation. Using an RNA protection assay (RPA) we show that the nucleotide where HIV-1 initiates transcription is variable. Our findings show that in both transiently transfected and chronically infected cells two major classes of HIV-1 transcripts beginning with G454 (3G-form) and G456 (1G-form) are present. Minor amounts of the 2G-form (beginning from G455) can also be seen in the cells. However, only 1G-RNA selectively packages into virions. Moreover, when the HIV-1 TSS is modified by mutagenesis so that the 1G-form becomes a minor RNA subspecies in the cells, this form is still preferentially packaged into virus particles. Sucrose gradient fractionation of the chronically infected cell cytoplasm revealed enrichment of the polysomal fraction with HIV-1 3G and 2G RNAs, suggesting that these RNA forms are preferentially translated.

T67. Insights into HIV-1 Proviral Transcription from an Integrative Structure of the Tat:AFF4:P-Tefb:TAR Complex

Ursula Schulze-Gahmen[1], Ignacia Echeverria[2], Goran Stjepanovic[1], Yun Bai[1], Huasong Lu[1], Dina Schneidman-Duhovny [2], Jennifer A. Doudna[1,3,4,5], Qiang Zhou[1], Andrej Sali[2], and James H. Hurley[1,5]

[1]Department of Molecular and Cell Biology and California Institute of Quantitative Biosciences, University of California, Berkeley, Berkeley, United States; [2]Department of Bioengineering and Therapeutic Sciences, Department of Pharmaceutical Chemistry, and California Institute of Quantitative Biosciences, University of California San Francisco, San Francisco, United States; [3]Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, United States; [4]Department of Chemistry, University of California, Berkeley, Berkeley, United States; [5]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, United States

HIV-1 Tat hijacks the human superelongation complex (SEC) to promote proviral transcription. HIV-1 Tat forms a physical complex with P-TEFb (Cyclin T1 and CDK9), AFF1/4, and HIV-1 TAR RNA. Solution structures of peptide:TAR complexes and a crystal structure of Tat:AFF4:P-TEFb are known, but the structure of the active Tat:AFF4:P-TEFb:TAR complex is not. We used hydrogen-deuterium exchange, small angle x-ray scattering, and selective 2'-hydroxyl acylation analyzed by primer extension to determine the integrative structure of the complex. The structure reveals direct contacts between helix α2 of AFF4 and TAR, along with contributions of Cyc T1 TRM and Tat ARM to TAR major groove binding. Point mutations in helix α2 of AFF4 reduced TAR binding to Tat-AFF4-P-TEFb in solution, and the corresponding mutations in AFF1 reduce Tat transactivation in cell-based reporter assays. These findings provide a structural framework for a critical complex in the regulation of HIV-1 latency.

T68. Probing Structural Dynamics and Kinetics of HIV-1 RNA Recognition to Guide RNA Drug Design

Nicole Orlovsky, Isaac Kimsey, Terrence Oas, and Hashim M. Al-Hashimi

Department of Biochemistry, Duke University, Durham, NC

Since the discovery of non-coding RNAs as key players and regulators of many biological processes and disease states, an increasing effort has been geared toward targeting RNA structure with small molecules for therapeutics. In particular, the HIV-1 transactivation response element (TAR) RNA has been at the focus of RNA drug discovery aimed at disrupting the TAR-Tat protein interaction that is essential for full-length transcription elongation and proliferation of the virus. However, few of these efforts have resulted in bioactive drugs with clinical potential, highlighting the need for further understanding of RNA-binding interactions to aid in effective drug design. In parallel, there is recent evidence that optimization of drug-target residence time may be important to drug efficacy, but this remains largely unexplored for RNA targets. Here, we apply a combination of NMR and fluorescence-based techniques to examine transient RNA-small molecule complexes, gaining insight into both the kinetics and thermodynamics underlying RNA recognition. Our approach enables simultaneous measurement of binding affinity, association and dissociation rates, as well as structural characterization of the ligand-bound RNA.

T69. Structure Based Methods to Target HIV-1 Transactivation Response Element RNA

Laura R. Ganser[1], Janghyun Lee[4], Bharathwaj Sathyamoorthy[5], Hal P. Bogerd[2], Yi Xue[1], Dawn K. Merriman[3], Aman Kansal[1], Paul Bieniasz[6], Bryan R. Cullen[2], Hashim M. Al-Hashimi[1]

[1]Department of Biochemistry, [2]Department of Molecular Genetics, and [3]Department of Chemistry, Duke University, Durham, NC 27710; [4]Department of Chemistry, Korea Advanced Institute of Science and Technology, Deejeon, 34141 South Korea; [5]Department of Chemistry, Indian Institute of Science Education and Research Bhopal, Bhopal 462-066, India; [6]Aaron Diamond AIDS ResearchCenter, Laboratory of Retrovirology and Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10016

Non-coding RNAs are vital to the regulation of many cellular processes, making RNA an attractive target for next-generation therapeutics. HIV-1 transactivation response element (TAR) RNA is one example of a non-coding RNA critical to cellular regulation and disease progression. TAR binds to the viral trans-activator protein, Tat, resulting in transcriptional enhancement necessary for viral replication and pathogenesis. There has been significant effort towards identifying small molecules that can block the TAR-Tat interaction, however, the limited number of successes highlights the importance of developing new methods to target RNA.

First, we describe parallel experimental and computational screening of >100,000 small molecules against TAR RNA followed by NMR screening of select compounds. The experimental high throughput screening (HTS) provided a rich data set with which we evaluated computational docking against a dynamic ensemble of apo-TAR RNA conformations previously determined by combining solution state Nuclear Magnetic Resonance (NMR) data and molecular dynamics simulations (Frank,2009; Stelzer, 2011). We find that ensemble-based docking predicts experimentally verified TAR-binding small molecules, including non-aminoglycosides, with an area under the curve (AUC) of the receiver operator characteristic (ROC) curve of AUC=0.92. This is compared to AUC=0.82-0.55 when docking against any one conformation. NMR chemical shift mapping experiments qualitatively support the conformational preferences of small molecules predicted by docking. Thus, ensemble-based docking can be applied to a flexible non-coding RNA target with accuracies comparable to those obtained from targeting well-folded proteins. However, preliminary cell-based studies did not reveal significant anti-viral activity for the small molecules.

As a novel approach to targeting RNA, we propose to target short-lived alternate conformations of TAR, termed excited states (ESs). TAR has two distinct excited states that are characterized by non-canonical mispairs that sequester residues necessary for the TAR-Tat interaction and that form unique pockets which may offer improved binding specificity. Preliminary mutational data suggests that stabilizing the ES2 of TAR prevents the TAR-Tat interaction *in vitro* and drastically reduces Tat-dependent trans-activation in cell-based assays. Further, we have generated a preliminary structural model of TAR ES2 using a combination of structure prediction and NMR chemical shifts of the ES and ES-stabilizing mutants. This model suggests that TAR ES2 has favorable and unique binding pockets. Future directions will include refining the ES2 TAR structural ensemble and carrying out virtual screening against it in search of molecules that stabilize this structure and have anti-viral activity.

T70. Virtual Screening Aided Design, Synthesis and SAR Study on Amiloride Derivatives as Probes for HIV-1 RNA

Umuhire Juru, A.[1], Patwardhan, N.N.[1], Eubanks, C.S.[1], Kapral G.J.[1], Lee, J. [2], Ganser, L.[2], Sathyamoorthy B.[2], Al-Hashimi, H. [2], and Hargrove, A.E.[1]

[1]Department of Chemistry, Duke University, Durham, NC; [2]Department of Biochemistry, Duke University School of Medicine, Durham, NC

The HIV-1 trans activation responsive element (TAR) RNA is found at the 5' end of every HIV-1 transcript and is essential for efficient HIV-1 transcription, a function that makes it an attractive drug target for HIV/AIDS. Using computational docking to an ensemble of HIV-1 TAR RNA structures, we have developed a TAR-targeted small molecule library based on the previously reported apical loop binder dimethyl amiloride (DMA). We have developed general synthetic routes for rapid, parallel synthesis of a series of diversified amiloride analogs by varying the substituents on two positions of the core pyrazine structure. A subset of these DMA analogs showed improved binding affinity for TAR relative to DMA, and SOFAST HSQC experiments revealed distinct binding modes for different library members. We are currently in the process of synthesizing a library of multivalent amiloride-based ligands, including dimers and the conjugation of other known RNA ligands. The resulting multivalent ligands will then be evaluated for binding against different HIV RNA targets. We expect the multivalent nature of these compounds to result in highly specific and high-affinity HIV RNA ligands.

F1. A “U2AF Homology Motif” of Tat-SF1, a Host Cofactor for HIV-1 RNA Splicing, Recognizes the Human SF3b1 Spliceosome Subunit and the HIV-1 Rev Protein

Steven Horner, Sarah Loerch, and Clara L. Kielkopf

Center for RNA Biology, University of Rochester School of Medicine & Dentistry, Rochester, NY 14642

Complex retroviruses such as HIV-1 co-opt the human spliceosome machinery for tightly coordinated production of their spliced mRNAs and genomic RNAs during the early and late stages of the viral life cycle. Tat-SF1 is a host protein that is critical in the production of fully spliced HIV-1 mRNAs in the early stage of the retroviral life cycle. Tat-SF1 is known to associate with the U2 small nuclear ribonucleoprotein subunit (snRNP) of the spliceosome and regulate the splicing of specific human transcripts. At present, the molecular mechanisms responsible for Tat-SF1 activities in human versus HIV-1 RNA splicing remain unknown.

Here, we show that Tat-SF1 recognizes the SF3b1 subunit of the U2 snRNP spliceosome particle. This sensitive SF3b1 subunit is frequently mutated in myelodysplastic syndromes and is targeted by natural and synthetic spliceosome inhibitors. We find that Tat-SF1 uses a subclass of RRMs called the “U2AF Homology Motif” (UHM) to interact with a “U2AF Ligand Motif” (ULM) of SF3b1. We determine the 1.1 Å resolution crystal structure of a Tat-SF1 UHM and 1.9 Å resolution structure of its SF3b1 ULM complex. We demonstrate a requirement for major interacting residues to form the complex by isothermal titration calorimetry (ITC) of structure-guided mutant proteins.

We further noted a ULM-like sequence in the HIV-1 Rev protein. Given the established importance of Tat-SF1 for HIV-1 replication, we hypothesized that the HIV-1 Rev could pre-empt Tat-SF1 for viral pathways *via* ULM–UHM interactions. To test this hypothesis, we tested the ability of the Rev ULM to bind Tat-SF1 by ITC. We find that the purified Rev ULM binds Tat-SF1 with higher affinity than an SF3b1 ULM.

Altogether, these results suggest a new mechanism of action for HIV-1 to pre-empt the human splicing machinery, in which the early stage HIV-1 Rev protein promotes late stage unspliced HIV-1 RNAs by disrupting the host Tat-SF1–U2 snRNP complex.

Supported by NIH R01 GM117005.

F2. Combined NMR and SAXS Studies of HIV and SIV Splicing Elements

Christopher E. Morgan, Niyati Jain, and Blanton S. Tolbert

Department of Chemistry, Case Western Reserve University, Cleveland, OH

The 9 kb HIV genome produces more than 40 mRNA transcripts through alternative splicing. Expression is regulated through interactions between host proteins and various splicing elements along the genome, which can serve to either enhance or silence splicing at a given splice site. In order to understand how alternative splicing is regulated in HIV, it is important to know the structures of the RNA splicing elements. Nuclear Magnetic Resonance (NMR) Spectroscopy and Small Angle X-ray Scattering (SAXS) are complementary structural methods that can be used to determine structures of protein and RNA in solution. Here, we present the structural studies of various HIV and SIV RNA splicing elements using both NMR and SAXS. Combining the two methods along with Molecular Dynamics (MD) simulations has increased the scope of both techniques. In that regard, NMR-SAXS studies have been used to model an SIV RNA stem loop, Stem Loop at Splicing Acceptor 1 (SLSA1), and are currently being utilized to determine the high-resolution structure of the 66 nucleotide Exon Splicing Silencer V (ESSV) stem loop and the 175 nucleotide Splice Site Acceptor 7 (ssA7) region of HIV. Collectively, the data presented here shows the ability of combined NMR-SAXS studies to determine structures of phylogenetically conserved HIV and SIV splicing elements.

F3. Splicing in a Panel of HIV-1 Transmitted/Founder Virus

Ann Emery[1] and Ronald Swanstrom[2]

[1]Genetics and Molecular Biology Curriculum, [2]Department of Biochemistry and Biophysics, University of North Carolina Chapel Hill

HIV-1 RNA is spliced to produce more than 40 different transcripts. Most HIV-1 splicing studies use NL4-3, a subtype B hybrid laboratory strain. We asked if splicing in NL4-3 is representative of splicing in clinical isolates, and if conserved splicing is critical for infection and spread of HIV-1. To address these questions we analyzed splicing in a panel of subtype B transmitted/founder virus.

We have adapted the PrimerID method to create an assay that quantifies HIV-1 RNA splicing patterns using deep sequencing. PrimerID is a random sequence tag in the cDNA primer, which is incorporated into a cDNA and all PCR products subsequently made from that tagged cDNA. Each cDNA gets a unique PrimerID and after sequencing, the PCR products can be sorted by their PrimerIDs. Each occurrence of a PrimerID is counted exactly once, and skewing in the PCR steps is filtered out. Our assay is a straightforward method to compare the relative abundance of different spliced transcripts within each size class of HIV-1 transcripts. We have also developed a program to identify cryptic splice sites.

We found that overall splicing structure was unchanged. In all isolates, the same acceptors and donors were used, but with a surprising range of variability. Other studies indicate that founder/transmitted virus optimize transmission at an initial fitness cost, but then lose that trait after establishing infection (Parrish 2013, Parker 2013). Balanced splicing may be a trait less critical to initial infection and so is sacrificed to accommodate mutations that optimize transmission. If so, we hypothesize that virus from these same patients at a later time point will revert to more tightly controlled splicing, and work on this question is ongoing.

Previous studies have suggested that cryptic splicing will be activated if/when usage of a regular acceptor is changed or blocked. Our data does not support this conclusion. Cryptic splicing remained negligible across the entire panel of transmitted/founder viruses, in spite of sequence changes that significantly changed acceptor and donor usage. In no case was a cryptic donor or acceptor activated above background levels. HIV-1 acceptors have been classified as weak, and clearly they diverge from the cellular splicing and branch point consensus sequences; however, they are still consistently recognized as major splice sites above the background noise. This suggests that many as yet unexplained factors contribute to HIV-1 splicing.

F4. RNA Specificity of HIV Splicing Factor Revealed by Global Analysis of its Binding Landscape

Niyati Jain, Hsuan-Chun Lin, Christopher E. Morgan, Michael E. Harris, and Blanton S. Tolbert

Case Western Reserve University

Heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) is an important RNA binding protein involved in many aspects of cellular and viral gene expression. HnRNPA1 is a known host factor required for controlling HIV genome splicing; however, the determinants of sequence specific interactions are poorly understood. Herein, we used high-throughput binding/sequencing (HITS-EQ) approach to perform global analysis of all possible sequence variants (n>16000) of the HIV ESS3 7-nt apical loop. The HITS-EQ results reveal that the N-terminal domain of hnRNPA1 binds ESS3 in a sequence and structure specific manner. The predominant and minimal specificity determinant observed through complete randomization of the 7-nt apical loop is 5'-AG-3'; however some 5'-AX-3' motifs constitute an enriched sub-signature identified from the landscape analysis. Nucleotide substitutions that reduce the size of the 7-nt apical loop by formation of the new WC base pairs are inhibitory to binding. Our findings therefore demonstrate that hnRNPA1 binds RNA targets with range of affinities, but specificity is decisively determined by the conformational availability of 5'AG-3' elements. These results provide important insights into the mechanism of interaction between hnRNPA1 and the HIV genome.

F5. Global Synonymous Mutagenesis Identifies Novel *Cis*-Acting RNA Sequences that Regulate HIV-1 Splicing and Replication

Matthew Takata[1,2], Steven Soll[1,2,3], Ann Emery[4,5], Daniel Blanco-Melo[1,2], Ronald Swanstrom[4,5] and Paul D. Bieniasz[1,2,3]

[1]Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY; [2]The Rockefeller University, Laboratory of Retrovirology, New York, NY; [3]Howard Hughes Medical Institute, Aaron Diamond AIDS Research Center, New York, NY; [4]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [5]Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

The HIV-1 genome contains RNA sequences that are multi-functional in that they both encode proteins and perform superimposed functions during viral replication. Known elements include splice donors, acceptors, and branch points; *cis*-regulatory elements that enhance or silence nearby splice sites; the Rev-response element; the central polypurine tract; the central termination sequence; GagPol ribosomal frameshift regulatory elements; and part of the packaging signal.

To determine whether there are additional, undiscovered *cis*-acting elements in the HIV-1 genome that are important for viral replication, we undertook a global silent mutagenesis strategy. A mutant, full-length HIV-1 sequence was designed that contained a maximum number of synonymous mutations in all open reading frames. However, all previously identified RNA elements were kept intact and no new AG or GT dinucleotides were added to avoid creating new splice donors and acceptors. The silent mutations were divided into 150-500 nucleotide blocks, which were synthesized and cloned to generate sixteen different mutant proviruses. Three of the mutants located in the central region of the genome (the open reading frames of Vif, Vpr, Tat, Rev, and Vpu) exhibited obvious splicing and replication defects. These splicing defects resulted from an overuse of existing splice sites in the HIV-1 genome. Single point mutations that arose following passage of these viruses restored replication competence by inactivating these overused splice sites. In order to understand which mutations introduced are responsible for the splicing defects we iteratively divided the mutants in half creating two new mutants. In some cases, the elements that control splice sites map to small determinants of ~30 nucleotides, while other elements could not be mapped to determinants that were smaller than ~150 nucleotides. Our data suggests that RNA elements of various types and replication of HIV-1 that have not been previously identified contribute to the proper balance of spliced HIV-1 mRNAs.

F6. Structure-Function Studies on the HIV-1 Rev Response Element

Ina O'Carroll[1,2], Yashna Thappeta[1], Lixin Fan[3], Edric Ramirez-Valdez[1], Sean Smith[1], Yun-Xing Wang[3], Alan Rein[1]

[1]HIV Dynamics and Replication Program, National Cancer Institute, Frederick, MD; [2] Chemistry Department, United States Naval Academy, Annapolis, MD; [3]National Cancer Institute, Structural Biophysics Laboratory, Frederick, MD

Nuclear export of the incompletely spliced HIV-1 genomic RNA is mediated by the viral Rev protein, which binds to the Rev-response element (RRE) on the HIV-1 RNA and, together with cellular factors, escorts unspliced viral messages from the nucleus to the cytosol. The secondary structure of the 351-nt RRE consists of a long domain (Domain I) crowned by several stem loops (Domains II-V). We recently reported the three-dimensional structure of the 233-nt "core" RRE in which each strand of Domain I is ~60 nt shorter than the full length sequence. The structure assumes an "A" shape and the domains are arranged such that the two known Rev-binding sites, on domains I and II, are positioned facing each other at an optimal distance for initial binding by a dimer of Rev. We suggested that this unique architecture explains why Rev binds selectively to RRE-containing RNAs within the nucleus, despite its ability to bind non-specifically to major grooves.

To elucidate the contributions of the individual domains to RRE function, we performed a detailed structure-function analysis. We found that: a) a truncation mutant in which Domain I is so short that the RRE is inactive can be "rescued" if the remaining base-pairs in the stem are replaced by GC base-pairs; this rescue is accompanied by a restoration of the A shape. This suggests that the long Domain I helps to maintain the overall RRE structure. b) Domains III-V are dispensable for moderate RRE activity, but only in the presence of a full-length Domain I; and c) when Domains III-V are deleted, RRE activity requires that the linker between Domains I and II be long enough to presumably bend, so that they can face each other. Maintaining the length of the linker but rendering it more rigid by replacing it with a run of A's also significantly reduces RRE activity. These results imply that a coaxial arrangement of Domain I and II (as in the popular "jellyfish model" of RRE action) is incompatible with activity. Together, the data support a model in which the complex "A" shape of the RRE is under strain, and is stabilized by Domain I; Domains III-V help position Domains I and II at an optimal distance to facilitate Rev binding.

F7. Role of DEAD-box Protein DDX1 in Assembly of Rev-RRE Nuclear Export Complexes

Rajan Lamichhane, John Hammond, Raymond Pauszek, Ingemar Pedron, Edwin van der Schans, James R. Williamson and David P. Millar

Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla CA 92037

The HIV-1 Rev (Regulator of Expression of Virion) protein activates nuclear export of unspliced and partially spliced viral mRNAs, which encode the viral genome and the genes encoding viral structural proteins. Rev interacts with a highly conserved region, the Rev Response Element (RRE), located within the viral mRNA. In order to activate nuclear export, multiple Rev proteins must assemble on the RRE. The host protein DDX1, a member of the DEAD box family, enhances the RNA export activity of Rev (Edgcomb, S. E. et al, J. Mol. Biol **415**, 61-74, 2012). We used a single-molecule fluorescence assay utilizing immobilized RRE (351 nt) and fluorophore-labeled Rev to monitor each step of Rev-RRE assembly, in the presence or absence of DDX1. More Rev monomers were observed to bind to the immobilized RRE in the presence of DDX1, indicating that DDX1 promotes oligomeric Rev-RRE assembly. To determine whether DDX1 acts through the Rev protein or the RRE RNA, both of which are known ligands for DDX1, we created DDX1 mutants that were unable to bind Rev (-Rev) or unable to bind the RRE (-RRE). Rev exhibited enhanced oligomerization behavior in the presence of the -Rev mutant, similar to WT DDX1, indicating that DDX1 does not need to associate with Rev in order to promote oligomeric Rev-RRE assembly. In contrast, only one or two Rev monomers bound to the RRE when the –RRE DDX1 mutant was present, fewer than observed with WT DDX1. This observation suggests that DDX1 must be capable of associating with the RRE in order to promote oligomeric Rev-RRE assembly. To determine whether Rev and DDX1 can simultaneously bind to the same RRE molecule, we carried out single-molecule FRET experiments with donor-labeled Rev and acceptor-labeled DDX1. Multiple FRET events were observed in the presence of immobilized RRE, confirming that Rev and DDX1 can occupy the same RRE molecule and that they are in close enough proximity for FRET to occur. In contrast, no FRET events were observed in the presence of acceptor-labeled –RRE mutant, confirming that FRET arises from binding of DDX1 to the RRE. Dwell-time analysis of the single-molecule trajectories revealed that DDX1 dissociates more rapidly from the RRE than Rev. Together, these results suggest that DDX1 interacts transiently with the RRE, producing an RNA conformation that promotes binding of multiple Rev monomers to the RRE. Supported by NIH P50 grant GM082545.

F8. Cryo-Electron Microscopy of HIV-1 RNAs

Zhaoming Su[1], Kaiming Zhang[1], Muyuan Chen[1], Sarah Keane[2], Jan Marchant[2], Steve Ludtke[1], Michael Schmid[1], Michael F. Summers[2], and Wah Chiu[1]

[1]National Center for Macromolecular Imaging, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030; [2]Howard Hughes Medical Institute and Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Cricle, Baltimore, Maryland 21250

Cryo-electron microscopy (cryo-EM) is a rapidly growing technology in structural biology, yet all samples studied by cryo-EM are on the order of 100 kDa and above. Here we present our cryo-EM structures of HIV-1 RNA fragments DIS dimer (~30 kDa) and RRE (~77 kDa). DIS is the crucial segment for the initiation of HIV-1 virion packaging, while RRE is responsible for exportation of unspliced and alternatively spliced HIV mRNAs out of the nucleus. Challenges of working with these HIV RNA samples are their inherent structural flexibility and relatively small sizes. We obtain a 17 Å EM map for DIS dimer and, in combination with NMR and MD simulation data, we are able to illustrate dynamics of different DIS conformations. Using cryo-EM we also generate a 20 Å RRE map with a well-defined topological structure. The overall shape of RRE remains the same under different ionic strengths and buffer conditions. Preliminary mutation of RRE reveals correlation between its secondary structures and our EM map. These results have provided fundamental insights into the dynamic behavior of small RNAs in solution, as well as posing significant new opportunities in RNA structural biology.

F9. A Combined Chemical and Phylogenetic Approach for HIV-1 and SIV RNA Secondary Structure Prediction Within and Among Infected Hosts

Brittany D. Rife[1,2], Carla N. Mavian[1,2], Susanna L. Lamers[3], David J. Nolan[1,2], Sergei Kosakovsky Pond[4], and Marco Salemi[1,2]

[1]Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL, USA; [2]Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA; [3]Bioinfoexperts, LLC, Thibodaux, LA, USA; [4]Department of Biology, Temple University, Philadelphia, PA, USA.

RNA structures encoded within lentiviral genomes are known to play important regulatory roles in viral replication, yet the majority of protein-coding RNA (cRNA) structures remained largely unexplored prior to recent full-genome structure analysis efforts using the chemical probing technique referred to as SHAPE (Watts et al., 2009; Pullom et al., 2013). Despite a powerful technique for *de novo* investigation of the RNA structural landscape, conserved RNA structure determination using this method is limited due to the inability to incorporate sequence and structural heterogeneity that are characteristic of RNA viruses such as HIV. A critical question still remains – what is the extent of this heterogeneity and how can we identify functionally relevant RNA structures? Coupling SHAPE with next generation sequencing (SHAPE-seq) can provide an in-depth scan of structure within diverse virus populations, but this type of experiment is costly and limited by the availability of biological specimens. Phylogenetic analysis offers a cheap and powerful alternative to garner critical insight into this heterogeneity and has been harnessed for several different *de novo* RNA structure determination programs. These programs have not been designed, however, to consider the biochemistry of complex interactions within large structural formations that encompass entire genes, and only one of these programs incorporates protein evolutionary constraints (RNA-Decoder). We have, therefore, proposed to combine these complementary approaches, using information from SHAPE analysis of a single reference sequence (HIV-1_{NL43} and $SIV_{MAC}239$) and phylogenetic (RNA-Decoder) analysis of subtype reference sequence alignments, to identify conserved HIV and SIV RNA structures within the envelope (*env*) gene, providing a higher resolution picture of the potential functionally relevant cRNA structure(s) within this region. Moreover, in order to determine if the environmental selective pressures within the host impact *env* RNA structure preservation during infection, we measured differences in evolutionary parameters, previously associated with structure conservation, between structure-based sequence partitions for HIV and SIV intra-host genealogies. Results of this study indicate potentially important local cRNA structures, such as nucleotides within the C2V3 junction, and establish a novel approach used to demonstrate cRNA structure preservation both within and among HIV/SIV-infected hosts. This combinatorial approach to *de novo* cRNA structure determination is currently being developed as a user-friendly program that utilizes single-site constraints based on user-specified threshold values of SHAPE reactivity and RNA-Decoder-derived pairing probabilities and is specifically optimized for the analysis of cRNA sequences.

F10. Impact of the HLA B*57 Allele on Intra-Host HIV-1 Capsid-Coding RNA Secondary Structure Diversity

Brittany D. Rife[1,2], Carla N. Mavian[1,2], Susanna L. Lamers[3], David J. Nolan[1,2], Frederick M. Hecht[5], Annika C. Karlsson[4], and Marco Salemi[1,2]

[1]Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL, USA; [2]Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA; [3]Bioinfoexperts, LLC, Thibodaux, LA, USA; [4]Karolinska Institutet, Stockholm, Sweden; [5]UCSF Positive Health Program, San Francisco General Hospital, University of California, San Francisco, California, USA

The HLA-B*5701 allele, which targets a particular region within p24, has been associated with control of viral replication (Norstrom *et al.,* 2012). For this reason, viral protein evolution in patients with this specific allele has been studied in order to provide insights into modes of protection that are essential for the successful development of vaccine and/or treatment strategies. Until recently, little research has been undertaken to explore the selective pressures at the level of both the protein and the underlying RNA secondary structure, the combination of which likely results in a complex interplay between protein and RNA structural evolution required for productive infection. In particular, since HIV-1-infected subjects usually harbor at any given time a heterogeneous population of viral strains the existence of a single RNA is unlikely. Based on this principle, we hypothesized that selective pressures at the protein level may affect the preservation of the HIV-1 RNA structural landscape within an infected host. Using the existing SHAPE structure (Watts *et al.,* 2009) as well as a variety of phylodynamic and RNA structural prediction methods, we investigated how HIV-1 diversity at the nucleotide and amino acid levels are associated with RNA structural diversity over the course of infection and the importance of the HLA-B*57 allele on RNA phenotypic (structural) evolution. Viral population dynamics were inferred utilizing extensive longitudinal sequence analysis with the Bayesian statistical framework for six HIV-1-infected patients with and six without the B*57 allele. Both RNA-Decoder and Mfold programs were then used to determine intra-host RNA structural diversity over time. Although no clear correlation was observed between changes in viral effective population size over time, a reflection of viral diversity, and temporal patterns in RNA secondary structure, patterns distinguishing patient cohorts were clearly evident. Specifically, subjects carrying the HLA-B*57 allele harbored significantly less stable structured RNA over the entire sampling time course (approximately 7 years post-infection). This allele-specific pattern may play an important role in the reduced viral replication observed within these patients and potentially prove useful as a therapeutic target.

F11. Conserved Global Structure and Function of Genomic RNA 5´-UTR Across Prototypic HIV-1 Subtypes

Roopa Comandur, Erik D. Olson, William A. Cantara, Joshua E. Hatterschide, Christopher P. Jones, and Karin Musier-Forsyth

Department of Chemistry and Biochemistry, Center for Retrovirus Research and Center for RNA Biology, The Ohio State University, Columbus, OH 43210

Human $tRNA^{Lys3}$ serves as the primer for reverse transcription in HIV-1. All three $tRNA^{Lys}$ isoacceptors are selectively packaged into virions through specific interactions between human lysyl-tRNA synthetase (hLysRS) and the viral Gag protein. We have proposed that tRNA primer release from hLysRS and targeting to the primer binding site (PBS) is facilitated by the interaction of hLysRS with a tRNA-like element (TLE) in the HIV-1 genome (*1*). In the HIV-1 NL4-3 isolate the TLE is located proximal to the PBS and contains a U-rich anticodon-like loop that resembles that of $tRNA^{Lys3}$. Small angle X-ray scattering (SAXS) analysis of the NL4-3 PBS/TLE domain has revealed that this region mimics the 3D structure of tRNA (*2*). Here, we study the 5´-UTR region of the HIV-1 MAL isolate, which contains a 23-nt insertion that is known to result in an alternative secondary structure relative to the NL4-3 isolate (*3*). Binding assays revealed high-affinity binding of hLysRS to a 229-nt MAL construct consisting of the PBS/TLE and Psi domains (K_D = 47 ± 13 nM). Truncation of the 229-nt construct to remove the Psi domain yielded a 98-nt construct with ~10-fold reduced binding affinity (K_D = 485 ± 133 nM). A similar trend was previously observed in the NL4-3 isolate (*1*). Binding to smaller stem-loop elements derived from the 98-nt construct was not observed and point mutations of this construct did not significantly affect binding. The global tertiary structure of both the 229-nt and the 98-nt MAL RNAs was analyzed using SAXS, revealing that these RNAs show remarkable structural similarity to analogous HIV-1 NL4-3-derived RNAs. The results suggest that tRNA mimicry is conserved across distinct HIV-1 isolates and that specific recognition of the HIV-1 genomic RNA PBS/TLE domain by hLysRS is largely based on overall 3D topology rather than sequence-specific recognition. The structural and functional similarities of the 5´-UTR suggest a conserved mechanism of HIV-1 primer release and targeting to the PBS among different isolates.

1. Jones, C. P., Saadatmand, J., Kleiman, L., and Musier-Forsyth, K. (2013) *RNA 19*, 219-229.
2. Jones, C. P., Cantara, W. A., Olson, E. D., and Musier-Forsyth, K. (2014) *Proc Natl Acad Sci USA 111*, 3395-3400.
3. Goldschmidt, V., Paillart, J. C., Rigourd, M., Ehresmann, B., Aubertin, A. M., Ehresmann, C., and Marquet, R. (2004) *J Biol Chem 279*, 35923-35931.

F12. HIV-1 Translation is Positively Regulated by Higher Order Conformation of 5'RNA

Ioana Boeras[1], Zhenwei Song[2], Xiao Heng[2], Sarah Monti[3], Aaron Rendahl[1], and Kathleen Boris-Lawrie[1]

[1]University of Minnesota, Saint Paul, MN; [2]University of Missouri, Columbia, MO; [3]University of Maryland, Baltimore County, Baltimore, MD

Structural information embedded in the 5'untranslated region (UTR) of viral and cellular RNAs critically regulates translation efficiency. Specific RNA structures of the HIV 5'UTR are critical to replication of the viral genome, but NMR and genetic studies demonstrate alternative 5'UTR conformations can constraint primary sequences necessary for intermolecular dimer-formation. An HIV RNA switch model has been promulgated to understand balanced regulation of mRNAs translated to viral structural proteins in balance with genomic RNA suitable for virion morphogenesis. To test the HIV RNA switch model in cells, we extended prior in-solution studies with synthetic HIV 5'UTRs to two quantitative cell-based assays measuring HIV translation during physiological post-transcriptional expression in human cells. Precise nucleotide-nucleotide (nt-nt) substitutions in 5'UTRs of HIV strains were demonstrated to energetically favor dimer conformation in-solution. Contrary to expectations, the cellular translation activity remained as robust as the native 5'UTR; dimer-prone 5'UTR did not attenuate gag translation, as previously observed in vitro, thus defying expectations that mRNA translation is negatively regulated by higher order structural complexity of the 5'UTR. Also, unexpectedly, nt-nt pairings that favored monomeric gag mRNAs significantly upregulated translation activity, thus defining a genetic signature to activate synthesis of lentiviral structural proteins. Our results demonstrate physiological distinctions predicted by an HIV RNA switch model in which a specialized translation RNP is recruited to monomer 5'UTRs. Our results demonstrate dimer-prone 5'UTR is suitable template for translation and spacio-temporally place translation regulation upstream and independent of RNA dimerization and genome assembly.

F13. Identification of the Extended Dimer Interface of the HIV-1 5′ Leader

Sarah C. Keane,[1] Verna Van,[1] Heather M. Frank,[1] Carly Sciandra,[1] Sayo McCowin,[1] Justin Santos,[1] Xiao Heng,[2] and Michael F. Summers[1]

[1]Howard Hughes Medical Institute (HHMI) and Department of Chemistry and Biochemistry, University of Maryland Baltimore County (UMBC). 1000 Hilltop Circle, Baltimore, MD 21250, USA; [2]Department of Biochemistry, University of Missouri, Columbia, MO, 65211, USA

Human immunodeficiency virus (HIV-1) selectively packages two copies of the full-length RNA genome. Packaging and dimerization are mediated by structural elements within the 5′ leader. A single stem-loop (DIS) containing a palindromic -GCGCGC- loop sequence has been shown to initiate the dimerization of two strands of RNA. Structural analysis of small RNA fragments corresponding to the DIS region resulted in the proposal of two different modes of dimerization: a kissing-loop dimer where the palindromic loops of two RNAs interact, and an extended-duplex dimer where the two stems participate in the intermolecular interface. However, the nature of the dimer interface within the context of the 5′ leader was unknown. We utilized a ^{2}H-edited nuclear magnetic resonance (NMR) approach to identify the dimer interface of the 5′ leader. Our data reveal that the dimeric 5′ leader contains an extensive dimer interface, which had not been predicted previously. We show that residues within DIS as well as the distal U5:AUG region participate in intermolecular base-pairing while the base pairing in TAR, Poly(A), PBS, and ψ is intramolecular. Additionally, we show that the formation of this extensive dimer interface occurs on a time-scale that is consistent with overall 5′ leader dimerization.

F14. Identifying Conserved RNA Binding Motifs for PSF/SFPQ, a Critical Host Factor for HIV-1 Replication

Gatikrushna Singh[1], Brittany Rife[2], Marco Salemi[2], Leslie Parent[3] and Kathleen Boris-Lawrie[1]

[1]University of Minnesota, Saint Paul, MN; [2]University of Florida, Gainesville, FL; [3]Penn State College of Medicine, Hershey, PA

As members of the NIH Center for HIV RNA Studies, we uncovered new information content embedded in the HIV 5' untranslated region (UTR). All retroviruses precisely balance post-transcriptional gene expression using cis-acting RNA motifs in the 5'UTR and catalytic ribonucleoprotein (RNP) machineries in cells. We identified cellular RNP components binding in common to HIV and C-type retrovirus 5'UTRs and undertook comparative analysis to discover cognate RNA binding sites for PSF/SFPQ, a critical regulator of lymphocyte biology. Using mutagenesis, RNA binding and cell-based assays we defined cognate GA-rich RNA structural motifs require PSF/SFPQ-binding activity to activate balanced expression of unspliced and spliced viral transcripts. Phylogenetic analysis of patient isolates identified conservation of the GA-rich motifs across HIV subtypes. Bits analysis of HIV and several other members of *Retroviridae* also demonstrated conservation of GA-motifs in consistent proximity to the 5' splice site. Site-directed mutagenesis of HIV and C-type spleen necrosis virus (SNV) 5'UTRs demonstrated necessity of the conserved GA-motifs to PSF/SFPQ binding. Cell-based assays demonstrated the same GA-mutations in HIV and SNV proviruses downregulated virion structural protein and virion precursor RNA. Quantitative RNA analysis identified the GA-rich PSF/SFPQ- binding motif is necessary for balanced expression of the HIV and SNV primary RNA. We speculate our identification of viral cis-sequence and cognate host factor necessary to biogenesis of structural protein and genomic RNP will provide a new strategy to attenuate retroviral infections.

F15. Renal Risk Variants of APOL1 RNA Contribute to Podocyte Injury by Activating Protein Kinase R

Koji Okamoto[1], Jason W. Rausch[2], Joon-Yong Chung[3], Avi Z. Rosenberg[4], Stephen M. Hewitt[3], Eisei Noiri[5], Stuart F.J. Le Grice[2], Maarten Hoek[6], Cheryl A. Winkler[7] and Jeffrey B. Kopp[1]

[1]Kidney Disease Section, NIDDK, NIH, [2]Reverse Transcriptase Biochemistry Section, Basic Research Program, FNLCR; [3]Experimental Pathology Lab, Laboratory of Pathology, Center for Cancer Research, NCI, NIH; [4]Department of Pathology, Johns Hopkins Medical Institutions; [5]Department of Nephrology, Endocrinology, Hemodialysis & Apheresis, University Hospital, The University of Tokyo; [6]Merck Research Laboratories, Merck and Co.; [7]Basic Research Laboratory, Center for Cancer Research, NCI, Leidos

African Americans have a four-fold increased lifetime risk for end-stage renal disease (ESRD), including that caused by HIV-associated nephropathy (HIVAN). Some of this major health disparity can be linked to genetic variants in APOL1, the gene encoding apolipoprotein L1, that are present only in individuals of African ancestry. Although this genetic association is well established, the mechanism by which APOL1 variants induce glomerular injury remains undefined. Here we show that in cultured cells, two APOL1 renal risk alleles activate protein kinase R (PKR), which orchestrates the cell stress response. This effect was preserved when APOL1 RNA was engineered to contain a premature stop codon, indicating that APOL1 protein was not required. This conclusion is further supported by clinical data indicating that APOL1-associated kidney diseases tend to progress to ESKD despite the use of anti-proteinuric and anti-inflammatory therapies. Structural analysis of truncated APOL1 RNAs provided evidence that the renal risk variants possess a secondary structure that may serve as a scaffold for tandem PKR binding and activation. Moreover, podocyte expression of the truncated, high risk allele RNAs in transgenic mice induced glomerular injury and proteinuria. Together, these findings provide a mechanism by which APOL1 variants damage podocytes and suggest novel therapeutic strategies that would involve inhibiting PKR or modulating its downstream targets.

F16. Structural Characterization of Large RNAs from HIV-1 Using NMR

Jan Marchant[1], Roald Teuben[1], Lindsay Glang[1], Geraldine Ezeka[1], Michael Lopresti[1], Kaiming Zhang[2], Zhaoming Su[2], Wah Chiu[2] and Michael Summers[1]

[1]Howard Hughes Medical Institute, University of Maryland Baltimore County; [2]Baylor College of Medicine

The application of NMR spectroscopy to the study of large, biologically relevant RNAs is complicated by a number of factors, including limited chemical shift dispersion, undesirable relaxation parameters and a relative lack of long-range dipolar contacts. We have developed a range of approaches designed to mitigate these difficulties, primarily based on the use of RNAs prepared with nucleotide- and atom-specific ^{2}H substitutions. Initially structural elements are identified from various combinations of ^{2}H-edited ^{1}H-^{1}H NOESY spectra and confirmed by comparison with appropriately designed oligonucleotide fragments, with further assignments facilitated by the production of differentially labeled chimera. NMR signal assignments and assignment validation are supported using database chemical shifts. Finally, high-resolution NMR ensembles can be combined with global structural information afforded by cryo-electron microscopy. We will present these approaches and current progress of their application to a 232 nucleotide minimal Rev response element from HIV-1.

F17. Development of Site-Specifically Labeled Nucleotides to Address Problems in NMR Spectroscopy

Andrew P. Longhini, Regan M. Leblanc, and Theodore K. Dayie

University of Maryland, College Park

Stable isotope labeling is central to NMR studies of nucleic acids, and the development of methods that incorporate isotope labels at specific atomic positions within each nucleotide promises to expand the size range and complexity of RNAs that can be studied by NMR. Combining enzymes from the pentose phosphate and other pathways with chemical synthesis of the ribose and base moieties, we have developed a streamlined chemo-enzymatic method to make nucleotides site specifically. This chemo-enzymatic approach is inexpensive, rapid and produces high yields of up to 90%. To demonstrate the range of applicability, we incorporated these custom made nucleotides into RNAs with sizes ranging from 27 to 59 nucleotides: A-Site (27 nt), the iron responsive elements (29 nt), a frame-shifting element from a human corona virus (59 nt), and fluoride riboswitch from *Bacillus anthracis* (48 nt), using standard *in vitro* techniques. Finally, we showcase the dramatic improvement in spectral quality arising from reduced crowding and narrowed linewidths, accurate and quantitative analysis of NMR relaxation dispersion (CPMG) and TROSY-based CEST experiments to measure μs-ms time scale motions, and an improved NOESY walk for resonance assignment.

F18. Variation in KSHV-Encoded microRNA Sequence Affect the Levels of Mature microRNAs in Kaposi Sarcoma Lesions

Vickie Marshall[1], Nazzarena Labo[1], Joanna Sztuba-Solinska[2], Elena M Cornejo-Castro[1], Karen Aleman[3], Kathleen M Wyvill[3], Lynne McNamara[4], Stuart FJ Le Grice[2], Robert Yarchoan[3], Thomas S Uldrick[3], Mark N Polizzotto[3], Patrick MacPhail[4] and Denise Whitby[1]

[1]AIDS and Cancer Virus Program, Leidos Biomedical, Frederick National Laboratory for Cancer Research, Frederick MD 21702; [2]Basic Research Program, National Cancer Institute, Frederick, MD 21702; [3]HIV and AIDS Malignancy Branch, National Institutes of Health, Bethesda, MD; [4]Clinical HIV Research Unit, Department of Internal Medicine, University of the Witwatersrand, Johannesburg, South Africa

Background: We previously reported Kaposi sarcoma-associated herpesvirus (KSHV) microRNA sequence variations in clinical samples correlated with increased risk of multicentric Castleman's disease (MCD). We demonstrated using *in vitro* systems that microRNAs with variant sequence have different secondary structure, maturation, and mature microRNA expression. In this study, we illustrate the association between microRNA sequence variation and changes in mature microRNA expression within Kaposi sarcoma (KS) lesions.

Methods: KSHV microRNA sequences were determined from 24 KS and 5 control skin biopsies from KSHV positive individuals. Mature KSHV microRNA expression was evaluated using 21 custom small RNA qPCR assays using RNA RNU6B as an endogenous control.

Results: Thirteen KSHV encoded microRNAs were detected at elevated levels in KS lesions compared to control biopsies. MicroRNA K12-9-5p was strongly down regulated in South African vs. US biopsies. Low expression of K12-9-5p was associated with single nucleotide polymorphisms (SNPs) in miR-K12-9-5p, 4-5p, 7-3p, and 3-5p. A SNP in miR-K12-3-5p resulted in down regulation of miR-K12-12-5p, 6-3p, and 8-3p and the upregulation of 5-5p. Sequence variations outside pre-microRNAs were also associated with changes in mature microRNA expression.

Conclusions: The levels of mature KSHV encoded microRNAs in KS lesions correlate with sequence variation reflecting changes in secondary and tertiary RNA structure.

F19. 5′ Start Site Heterogeneity of the HIV-1 RNA and its Effect on Structure and Function

Joshua Brown, Seungho Choi, Michael Lopresti, Hannah Carter, Aishwarya Iyer, Jana Hiji, Nicolas Bolden, Lindsay Glang, and Michael Summers

University of Maryland Baltimore County

The goal of this study is to establish how naturally occurring sequence heterogeneity and capping at the 5′-end of the HIV-1 genomic RNA influences its structure and function. All lentiviral genomes are transcribed in infected cells from an integrated proviral DNA that contains a stretch of three sequential guanosines, any of which could potentially serve as the transcription start site. Recently, Kawai and co-workers reported that cells infected with HIV-1 produce both 5´-capped HIV-1 genomes that begin with one or two guanosines (1G and 2G, respectively), that the 1G genome is specifically packaged into virions, and that 5´-end heterogeneity influences reverse transcription. In collaboration with Dr. Telesnitsky at the University of Michigan, we have obtained similar evidence that HIV-1 5´-end heterogeneity influences RNA fate. The 5´-capped 1G genomes were selected efficiently for packaging and the 2G genomes were enriched on polysomes, apparently preferred for translation and possibly for splicing. Preliminary data found evidence that 5´-end heterogeneity significantly influences the structure of the RNA *in vitro*. Native gel electrophoresis studies reveal that 5´-capped 1G leader RNAs readily form dimers, whereas 5´-capped 2G RNAs adopt stable monomers. I plan to further test this observation by solving the secondary structure using a recently developed nuclear magnetic resonance (NMR) approach that involves nucleotide-specific deuterated RNA prepared by *in vitro* transcription. Additional future work includes testing structural elements that appear to be important for regulating RNA splicing, transcriptional activation, and translation by *in vivo* mutagenesis experiments.

F20. DHX9/RHA Binding to the PBS-Segment of the Genomic RNA during HIV-1 Assembly Bolsters Virion Infectivity

Ioana Boeras[1], Zhenwei Song[2], Andrew Moran[2], Jarryd Franklin[2], William Clay Brown[3], Marc Johnson[4], Kathleen Boris-Lawrie[1] and Xiao Heng[2]

[1]Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN 55108, USA; [2]Department of Biochemistry, University of Missouri, Columbia, MO 65211, USA; [3]Center for Structural Biology, Life Sciences Institute, University of Michigan, Ann Arbor, MI 48109, USA; [4]Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO 65211, USA

Cellular RNA binding proteins incorporated into virions during HIV-1 assembly promote the replication efficiency of progeny virions. Despite its critical role in bolstering virion infectivity, the molecular basis for incorporation of DHX9/RNA helicase A (RHA) to virions remains unclear. Here, cell-based experiments demonstrate truncation of segments of the HIV-1 5´-untranslated region (5´-UTR) that are distinct from the core encapsidation sequence, eliminated virion incorporation of RHA, indicating that RHA recruitment is mediated by specific interactions with the HIV-1 5´-UTR. In agreement with biological data, isothermal titration calorimetry determined the dimer conformation of the 5´-UTR binds one RHA molecule per RNA strand, and the interaction is independent of nucleocapsid protein binding. Nuclear magnetic resonance spectra employing a deuterium-labeling approach enabled resolution of the dimeric 5´-UTR in complex with the RHA N-terminal domain. The structure of the large molecular mass complex was dependent on RHA binding to a double-stranded region of the primer binding site (PBS)-segment of the 5´-UTR. A single A to C substitution was sufficient to disrupt biophysical conformation and attenuate virion infectivity in cell-based assays. Taken together, our studies demonstrate the structural basis for HIV-1 genomic RNA to recruit beneficial cellular cofactor to virions. The support of progeny virion infectivity by RHA is attributable to structure-dependent binding at the PBS-segment of HIV-1 5´-UTR during virus assembly.

F21. RNA Structure Provides Insights into Mechanism of Selective Genome Packaging by Retroviral Gag

Erik D Olson[1], Tiffiny Rye-McCurdy[1], Brian R Thompson[1], Ioulia Rouzina[1], Leslie Parent[2], and Karin Musier-Forsyth[1]

[1]Department of Chemistry and Biochemistry, Center for RNA Biology, and Center for Retroviral Research, The Ohio State University, Columbus, OH; [2]Penn State College of Medicine, Departments of Medicine and Microbiology and Immunology, Hershey, PA

In infected cells, host RNA is present in vast excess to viral RNA, yet all retroviruses specifically package their genomic RNA (gRNA) as a dimer. The Psi element within the 5´-untranslated region (5´UTR) is critical for gRNA packaging through interaction with retroviral Gag. Gag is a precursor protein made up of the matrix (MA), capsid, and nucleocapsid (NC) domains. However, under physiological conditions, Gag binding affinity for Psi versus non-Psi RNAs is not significantly different. To investigate the mechanism of this selectivity, salt-titration binding assays were performed, allowing us to determine two binding parameters: $K_{d(1M)}$, which describes the strength of the non-electrostatic component of binding, and Z_{eff}, the number of charge contacts that mediate the protein-RNA interaction. Compared to other RNAs tested, HIV-1 Gag binds to Psi RNA with lower $K_{d(1M)}$ and Z_{eff}, indicative of a highly specific interaction. In contrast, Gag bound a non-Psi RNA with a weaker $K_{d(1M)}$ and a significantly higher Z_{eff}, suggesting another domain of Gag interacted with this RNA. A Gag mutant lacking MA bound both Psi and non-Psi RNA with a more similar $K_{d(1M)}$ and Z_{eff} values that were indistinguishable, suggesting that the MA domain plays a role in Gag's discrimination between Psi and non-Psi RNA by preferentially interacting with non-Psi RNAs. We have also investigated the elements of Psi that contribute to the specific HIV-1 Gag interaction. We find that there is a cluster of interaction sites near the junction of stem loops 1 and 3 of Psi that are critical for inducing Gag's specific binding mode, and that dimerization is important only in the context of longer Psi RNA constructs.

We have extended our salt titration studies to Simian immunodeficiency virus (SIV) and Rous sarcoma virus (RSV). Similar to HIV-1, we found that RSV Gag was able to effectively discriminate RSV Psi and non-Psi RNA by interacting with non-Psi RNA in a less specific binding mode and by engaging the MA and NC domains. In contrast, whereas SIV NC recognizes HIV-1 Psi in a specific manner, specific binding to SIV Psi is not observed by any NC or Gag tested. Small-angle X-ray scattering (SAXS) analysis of HIV-1 and RSV Psi RNAs revealed a remarkably similar global fold. In contrast, the putative SIV Psi RNA adopted a significantly different fold, potentially explaining the lack of specific protein binding. Based on these data, we hypothesize that specific RNA structures modulate Gag's binding mode, contributing to selective gRNA packaging.

F22. Probing HTLV-1 Matrix-Viral Genomic RNA Interactions

William A. Cantara, Weixin Wu, and Karin Musier-Forsyth

Department of Chemistry and Biochemistry, Center for RNA Biology, and Center for Retroviral Research, The Ohio State University, Columbus, OH 43210

During assembly of retrovirus particles, the Gag polyprotein selectively packages two copies of full-length viral genomic RNA (gRNA) in a process mediated by a specific interaction between Gag and the gRNA packaging signal, Psi. In HIV-1, the nucleocapsid (NC) domain of Gag is critical for specific gRNA selection. By comparison, the mechanism of gRNA packaging in deltaretroviruses (e.g., BLV, HTLV-1 and HTLV-2) is less clear. Previous studies showed that both BLV matrix (MA) and NC are involved in gRNA packaging. In addition, HTLV-2 MA binds nucleic acids (NA) with higher affinity than HTLV-2 NC and is a more robust NA chaperone protein. We have proposed that deltaretroviral MA plays a more important role than NC in the initial gRNA selection process. Here, we focus on HTLV-1 MA-gRNA recognition. Fluorescence anisotropy binding assays reveal that HTLV-1 NC binds to a non-specific 20-mer DNA oligonucleotide (K_d=230 nM), but fails to bind to RNA stem loops SL1 and SL2 derived from the putative HTLV-1 gRNA packaging signal. In contrast, MA binds with moderate affinity to ssDNA, SL2 and SL1-SL2 (K_d=1.2±0.3 μM, 2.0±0.1 μM and 1.5±0.3 μM, respectively); very weak binding was observed to SL1 alone. Salt-titration experiments showed that HTLV-1 MA binding to these small RNA constructs is not as specific as HIV-1 NC binding to its packaging signal. These results suggest that HTLV-1 MA may require additional gRNA sequence elements for specific recognition. We investigated HTLV-1 MA binding to four large HTLV-1 gRNA constructs: 5'UTR (nt 1- 625), R-U5 (nt 1-404), U5-5'gag (nt 228-449), and 5'gag (nt 450-625). Gel shift assays reveal that MA binds to all four constructs with similar binding affinity (K_d ~2 μM). Selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) probing was used to determine the secondary structure of the HTLV-1 5'UTR and to map HTLV-1 MA binding sites. To facilitate SHAPE probing data analysis, a user-friendly tool was developed that improves the speed while maintaining the fidelity of analysis. SHAPE probing did not support the formation of the previously proposed SL1. Significantly *decreased* reactivity was observed upon MA binding in three regions: a region upstream of the primer-binding site (PBS), the PBS region, and SL2, which was designated as the "new SL1" and contains a palindromic sequence in the apical loop. Significantly *increased* reactivity was observed in the PBS region and in regions within and proximal to the new SL1, indicating MA-induced conformational changes of gRNA. Our results show that HTLV-1 MA interacts with specific regions in the HTLV-1 5'UTR and may play a role as a NA chaperone protein, destabilizing thermodynamically stable secondary structures in the PBS domain.

F23. Cellular MicroRNAs are Packaged into HIV-1 Virions

Hal P. Bogerd[1], Edward M. Kennedy[1], Adam W. Whisnant[2] and Bryan R. Cullen[1]

[1]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710, USA; [2] Institute of Virology, University of Würzburg, Würzburg, Germany

Using small RNA deep sequencing we have demonstrated that highly purified HIV-1 virions produced from the human T-cell line CEM-SS incorporate cellular miRNAs. Moreover, a subset of these miRNAs appears to be selectively enriched in virions relative to their cellular levels, and among these were several miRNAs (miR-155, miR-432-3p and miR-186) previously shown to target RISC to the HIV-1 viral RNA using the PAR-CLIP (Photoactivatable Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation) technique. The insertion of artificial targets for endogenous or ectopically expressed miRNAs into the *nef* region of HIV-1 resulted in efficient incorporation of miR-92a and miR-155 into virions, and a reproducible inhibition of viral replication. This inhibition was modest when imperfect miRNA targets were inserted into HIV-1, and significantly more pronounced with perfect targets, in both the HEK293T and CEM-SS cell lines. However, since the ablation of miRNA biogenesis by inactivation of Dicer does not enhance HIV-1 replication or gene expression, the functional importance of the incorporation of cellular miRNAs into wild-type HIV-1 virions is presently unclear.

F24. In Vitro Selective Binding between the HIV-1 Packaging Signal and Gag is Driven by a Delicate Balance between Specific and Non-Specific Interactions

Mauricio Comas-Garcia[1], Siddhartha A.K. Datta[1], Rajat Varma[2] and Alan Rein[1]

[1]HIV DRP, National Cancer Institute, Frederick, MD 21702, USA; [2]Laboratory of Systems Biology, National Institute of Allergic and Infectious Diseases, Bethesda, MD, 20892

The assembly of retroviruses depends on a delicate balance between protein-protein and protein-RNA interactions. Virion assembly is mostly driven by protein-protein interactions, while selective packaging of the genomic RNA depends (gRNA) on specific protein-RNA interactions. In HIV-1 selective packaging is thought to be driven by binding of Gag to a high-affinity binding site in the gRNA (*packaging signal* [Ψ]). A great deal of work has focused on identifying the Ψ, as well as on measuring the strength of Gag/Ψ interactions; however, the molecular mechanism of selective packaging of the HIV-1 gRNA it is still not clear. Most *in vitro* studies have shown that although Gag has a nanomolar affinity for the Ψ, it can bind also to other RNAs with similar affinities. This suggests that an exclusively high-affinity binding mechanism is not sufficient to explain *in vivo* packaging selectivity.

We have used Fluorescence Correlation Spectroscopy to measure Gag/RNA interactions in the absence of virus-like-particle assembly. We show that Gag/RNA binding is cooperative and that Gag condenses RNA (RNA condensation by capsid proteins has been reported for other viruses). By using WM-Gag (which cannot dimerize) we show that Gag-Gag interactions are essential for RNA condensation. Interestingly, when Gag-Gag interactions are weak the affinity for the non-Ψ RNAs is greatly reduced, while the affinity for the dimeric Ψ remains unchanged. In agreement with a previous report (Webb et al. RNA 2013) we find that binding to ψ is far more salt-resistant than binding to other RNAs. Thus there is a significant difference between the binding to Ψ and to the control RNAs, even though, because of the nonspecific interactions, overall affinities are similar at physiological ionic strengths. This difference in affinities also becomes apparent in binding measurements in the presence of tRNAs or with Gag mutants impaired in MA/RNA or Gag-Gag interactions. The contributions of MA/RNA and Gag-Gag interactions are considerably greater for the control RNAs than for dimeric Ψ. Furthermore, at physiological salt and in the absence of MA/RNA and Gag-Gag interactions the dimeric Ψ exhibits high affinity for Gag, while binding to the control RNAs is severely impaired.

Finally, our results suggest that Gag-Gag interactions are stronger when bound to the dimeric Ψ than to the control RNAs. If binding to ψ enhances Gag-Gag interactions, then a Gag/Ψ complex could act as a nucleation site for virion assembly. We are currently investigating if packaging selectivity can be explained through this mechanism.

F25. HIV-1 Gag Co-Localization with Unspliced vRNA in the Nucleus Occurs During or Shortly After Transcription

Kevin M. Tuffy[1], Rebecca J. Kaddis Maldonado[1], Breanna Rice[1], Alan Cochrane[3], and Leslie J. Parent[1,2]

[1]Departments of Medicine and [2]Microbiology & Immunology, Penn State College of Medicine, Hershey, PA; [3]Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada

The retroviral Gag protein selects the unspliced viral RNA for encapsidation as genome into new virions. It was initially believed that Gag first interacted with genomic RNA in the cytoplasm before trafficking of the Gag-RNA complex to the plasma membrane. However, data from our laboratory demonstrates that the nuclear trafficking of the Rous sarcoma virus (RSV) Gag protein near sites of transcription is linked to efficient genome packaging. Furthermore, using subcellular fractionation, we observed the enrichment of Gag in chromatin-bound protein fractions. Together, these data suggest that RSV Gag is tethered in the peri-chromatin space near sites of RNA synthesis where it selects its genomic RNA for packaging. In addition to RSV Gag, the Gag proteins of other retroviruses have also been shown to traffic through the nucleus (e.g., PFV, FIV, MPMV, MMTV, HIV-1), although the role of Gag nuclear trafficking in the replication of these viruses are poorly understood.

We are currently investigating whether the nuclear localization of HIV-1 Gag has a role in RNA packaging similar to that of RSV Gag. To examine this possibility, we utilized an inducible replication-deficient provirus that expresses a Gag-GFP fusion protein and performed fluorescence in situ hybridization to detect unspliced viral RNA. We visualized HIV-1 Gag and unspliced viral RNA complexes in the nucleus using confocal microscopy and performed quantitative colocalization analysis. We observed that HIV-1 Gag specifically co-localized with a subset of unspliced viral RNA within distinct foci in the nucleus. Inhibition of transcription with DRB or actinomycin D led to a decrease in the total amount of nuclear HIV-1 Gag and unspliced viral RNA foci, although there was a significant increase in the degree of nuclear Gag-unspliced viral RNA co-localization. However, inhibition of unspliced viral RNA export by treatment with the CRM-1 inhibitor LMB increased the accumulation of Gag and unspliced viral RNA in the nucleus but did not alter the amount of Gag-unspliced viral RNA co-localization in the nucleus. Together these data suggest that a subpopulation of nuclear HIV-1 Gag associates with unspliced viral RNA during or shortly after transcription. There appears to be a narrow window of opportunity during which Gag-viral RNA association occurs, and once the viral RNP is export competent, Gag can no longer be added to viral RNA complexes. The increase in the number of Gag and unspliced viral RNA foci with LMB treatment suggests that HIV-1 Gag nuclear export may be linked to Rev function since Gag itself does not appear to contain a CRM1-dependent nuclear export signal.

F26. Readily Accessible Multiplane Microscopy: 3D Tracking the HIV-1 Genome in Living Cells

Michelle S. Itano, Marina Bleck, Daniel S. Johnson, and Sanford M. Simon

Laboratory of Cellular Biophysics, The Rockefeller University, New York, NY

HIV-1 infection and the associated disease AIDS are a major cause of human death worldwide with no vaccine or cure available. The trafficking of HIV-1 RNAs from sites of synthesis in the nucleus, through the cytoplasm, to sites of assembly at the plasma membrane are critical steps in HIV-1 viral replication, but are not well characterized. Here we present a broadly accessible microscopy method that captures multiple focal planes simultaneously, which allows us to image the trafficking of HIV-1 genomic RNA with high precision. This method utilizes a customization of a commercial multichannel emission splitter that enables high-resolution 3D imaging with single-molecule sensitivity. We show with high temporal and spatial resolution that HIV-1 genomic RNAs move diffusively, moving fastest in the cytosol, and undergo confined mobility at sites along the nuclear envelope, nucleus and in the nucleolus. These provide important insights regarding the mechanism by which the HIV-1 RNA genome is transported to the sites of assembly of nascent virions.

F27. Biochemical Reconstitution of Selective HIV-1 Genome Packaging

Lars-Anders Carlson[1], Yun Bai[1,3], Sarah C. Keane[2], Jennifer A. Doudna[1], and James H. Hurley[1]

[1]Department of Molecular and Cell Biology, University of California, Berkeley; [2]University of Maryland, Baltimore County; [3]Current address: School of Life Science and Technology, ShanghaiTech University, Shanghai, 201210, China

HIV-1 Gag selectively packages the genomic viral RNA into assembling virus particles in the context of a large excess of cytosolic human RNAs. As Gag assembles on the plasma membrane, the HIV-1 genome is enriched relative to cellular RNAs by a mechanism which is incompletely understood. We used a minimal system consisting of purified RNAs, recombinant myristylated HIV-1 Gag and giant unilamellar vesicles to recapitulate the selective packaging of the 5' untranslated region of the HIV-1 genome in the presence of excess competitor RNA. In this assay, the Rev-responsive element and a non-viral control RNA were not selected for to the same degree. Mutations in the CA-CTD domain of Gag which subtly affect the self-assembly of Gag on membranes abrogated RNA selectivity. We further found that tRNA suppresses Gag membrane binding less when Gag has bound viral RNA. The ability of HIV-1 Gag to selectively package its RNA genome and its self-assembly on membranes are thus interdependent on one another.

F28. Screening Potential Small Molecule Inhibitors Against the Core Encapsidation Signal of HIV Using Nuclear Magnetic Resonance

Julie Nyman, Jessica Zaki, and Michael Summers

UMBC, Department of Chemistry and Biochemistry

The human immunodeficiency virus (HIV) causes prevalent health issues as it infects more than 1.2 million people in the United States. While current HIV drugs target proteins involved in various steps in the viral life cycle, the search for new drug targets and for more effective treatments is ongoing. HIV's RNA genome contains the highly conserved region called the core encapsidation signal (CES). The CES RNA allows for the packaging of the genome into new virions. Because the CES is highly conserved, it is an attractive drug target. Nuclear magnetic resonance (NMR) can be used to characterize the interactions between the CES and potential small molecule inhibitors. In this way, we will be able to determine if an inhibitor is likely to improve current HIV therapies.

F29. Characterizing the Host Cell Factors Involved in HIV-1 Gag Trafficking to Sites of Virus Assembly

Rachel Van Duyne, Philip R. Tedbury, and Eric O. Freed

Virus-Cell Interaction Section, HIV Dynamics and Replication Program, NCI-Frederick, MD

HIV-1 particle assembly and release require the coordinated effort between cellular and viral proteins, a process driven primarily by the HIV-1 Gag polyprotein. In the late stages of the viral replication cycle, the Gag precursor, Pr55Gag, is synthesized in the cytoplasm, cotranslationally myristylated, and recruited to the plasma membrane (PM) through binding to the phospholipid $PI(4,5)P_2$, associating Gag with the PM. Though the mechanisms of virus release are well studied, the trajectory by which Gag traffics to the site of virus assembly remains one of the least understood aspects of HIV-1 replication. We previously determined that overexpression of the Golgi-localized γ-ear containing Arf-binding (GGA) proteins severely reduces HIV-1 particle production by impairing Gag trafficking to the membrane through disruption of Arf (ADP-ribosylation factor) protein activity. We therefore aim to characterize the role of the Arf family of vesicular trafficking proteins in the context of HIV-1 assembly and release. At levels non-toxic to cells, overexpression of WT Arf proteins has little to no effect, however expression of dominant-negative Arf1 (Arf1DN) potently inhibits HIV-1 release efficiency, Env processing, and particle infectivity. Interestingly, expression of Arf3DN does not confer the Arf1DN-mediated phenotype, despite greater than 96% amino acid sequence identity between these two Arf family members. We demonstrate that Arf1DN is able to inhibit virus release independent of MA and Nef and can also inhibit HIV-1 mutants defective in budding, maturation, and membrane targeting. We also observed that Arf1DN does not result in the mis-targeting or sequestering of Gag to intracellular sites of assembly. Interestingly, expression of Arf1DN has been implicated in the complete disassembly of the Golgi, suggesting that Arf1DN-mediated inhibition of virus release may occur at very early steps of Gag trafficking and may involve components of Golgi function. We are currently determining if the Arf1DN-mediated disruption of Gag trafficking results in the accumulation of Gag at the Golgi or at intracellular membranes. Elucidating the HIV-1 Gag trafficking pathway is significant for both the comprehensive understanding of HIV-1 biology and the identification of novel targets for antiretroviral inhibitors.

F30. Retroviral Gag Puncta Biogenesis and Quantitative Measurements of Gag Stoichiometry

Isaac Angert, John Eichorst, Jessica L Martin, Wei Zhang, Louis M Mansky, and Joachim D Mueller

Institute for Molecular Virology, University of Minnesota, Minneapolis, MN 55455

The Gag polyprotein is the essential retrovirus structural protein required for the assembly and release of retrovirus particles. Much of our knowledge regarding Gag biology has been focused on a limited number of retroviral systems. One understudied retrovirus, in this regard, is human T-cell leukemia virus type 1 (HTLV-1), a deltaretrovirus that causes an adult T-cell leukemia/lymphoma. In this study, we have 1) investigated, via comparative analysis with HIV-1, the pathways by which HTLV-1 Gag is recruited to Gag puncta at the plasma membrane by use of Gag fused to the photoconvertible fluorescent protein mEos2, and 2) developed a 2-photon imaging technique for measuring Gag stoichiometry at single particle resolution. We have used total internal reflection microscopy (TIRFm) to selectively photoconvert Gag-mEos2 present at the plasma membrane. In particular, we have used the fluorescence emission of mEos2, which changes from green to red after being exposed to a pulse of violet light. Color changes to Gag puncta help to reveal the location (membrane vs. cytoplasm) where Gag was recruited from during Gag puncta biogenesis red fluorescence is indicative of Gag being recruited from the plasma membrane, while green fluorescence is indicative of Gag being recruited from the cytosol. Measurements were made by using two complementary modalities: 1) particle tracking, which allowed for monitoring the recruitment and growth kinetics of puncta, and 2) time-lagged monitoring over a time period of fifteen minutes, which eliminates many technical difficulties (such as photobleaching) that arise in particle tracking experiments. The results support the conclusion that HTLV-1 Gag was recruited primarily from the plasma membrane, while HIV-1 Gag was primarily recruited from the cytosol. Retrovirus particles are known to be heterogeneous in regards to their size and Gag stoichiometries. Our previous analyses have used fluorescence fluctuation spectroscopy (FFS) methodologies to measure average Gag copy number per particle while suppressing population heterogeneity. Here, we have developed a 2-photon imaging technique in order to directly measure the absolute Gag copy number with single particle resolution. FFS measurements of monomeric fluorescent label brightness were used to convert the intensity of each virus-like particle into a number of monomers. Taken together, our findings provide new insights into 1) the distinct differences that exist regarding HTLV-1 and HIV-1 Gag puncta biogenesis at the plasma membrane; and 2) quantitation of Gag stoichiometry in HTLV-1 and retrovirus particles.

F31. HTLV-1 and HIV-1 CA-CA Interactions Involved in Virus Particle Assembly

Jessica L Martin, Rachel Marusinec, Louis M Mansky

University of Minnesota, Institute for Molecular Virology, Minneapolis, MN

The retroviral Gag protein is the main structural protein responsible for virus particle assembly and release. The Gag protein alone in most instances is sufficient for production of virus-like particles (VLPs). The Gag proteins of human immunodeficiency virus type 1 (HIV-1) and human T-cell leukemia virus type 1 (HTLV-1) both have structurally conserved capsid (CA) domains, sharing a β-hairpin turn and a centralized coiled-coil-like structure of six α helices in the CA amino-terminal domain (NTD) as well as four α helices in the CA carboxy-terminal domain (CTD). For both retroviruses, CA drives Gag oligomerization, which is critical for the formation of an immature Gag lattice structure requisite for the formation of VLPs.

It has previously been shown that the HIV-1 CA CTD is a primary determinant for CA-CA interactions. A previous study with HTLV-1 suggested roles for both the CA NTD and CA CTD in Gag-Gag interactions and particle formation. In this study, we sought to more clearly define the role(s) of the HTLV-1 CA CTD and CA NTD in Gag-Gag interactions and in particle release. To do this, we first created a panel of four chimeric Gag constructs in which the CA subdomains were interchanged between HIV-1 and HTLV-1, including HIV-1 Gag with the HTLV-1 CA CTD (HIV-HT-CTD) and HTLV-1 Gag with the HIV-1 CA NTD (HTLV-HI-NTD). The subcellular distribution and protein expression levels of the chimeric Gags were analyzed by confocal microscopy and immunoblotting, respectively. The morphologies of VLPs were also investigated by cryo-electron microscopy. The subcellular distribution of HTLV-HI-NTD and HIV-HT-CTD Gag proteins appeared diffuse and non-punctate, and VLP production was abrogated. Our observations indicated that the HIV-1 CA CTD could functionally replace the HTLV-1 CA CTD, but that the HIV-1 CA NTD could not functionally replace the HTLV-1 CA NTD.

To further investigate the HTLV-1 CA NTD, we conducted alanine-scanning mutagenesis within the loop domains. Site-directed mutants were screened for diffuse vs punctate Gag localization in cells as well as particle release, and this analysis identified 3 groups of mutants. Group 1 had WT phenotypes; Group 2 mutants had a diffuse localization in cells but produced particles; Group 3 mutants had a diffuse Gag localization in the cytoplasm but did not produce particles. Six key mutants (CAs containing alanines at residues 16-17, 17-18, 46-47, 47-48, 131-132, and 132-133) were identified in Group 3 and were further analyzed using *in vitro* methods. Specifically, we purified the HTLV-1 CA and analyzed the ability of WT CA and mutants to form oligomers via crosslinking and immunoblot analyses. Taken together, we have found that while structural and functional similarities exist in the CA domain of HIV-1 and HTLV-1 Gag, the HTLV-1 CA NTD is distinct in that key residues required for Gag-Gag interactions are located in this domain.

F32. Design and Characterization of Enveloped Protein Nanoparticles

Jörg Votteler[1], Cassie Ogohara[2], Sue Yi[2], David Belnap[1], David Baker[2], Neil P. King[2], and Wesley I. Sundquist[1]

[1]CHEETAH Center and Department of Biochemistry, University of Utah, Salt Lake City, Utah 84113; [2]Institute for Protein Design, University of Washington, Seattle, Washington 98195

Retroviruses like HIV-1 assemble in producer cells and acquire their lipid envelopes as they bud through the plasma membrane. The Gag polyprotein contains the membrane binding, assembly and budding activities necessary to create extracellular virions, which can then transduce new cells and deliver their cargoes. These activities underlie the efficacy of retroviral vectors, which have been explored extensively as therapeutic delivery vehicles. There are, however, potential limitations, including safety concerns, immunogenicity and inefficient packaging of non-nucleic acid cargoes. To expand the toolbox of candidate delivery systems and to test our understanding of the fundamental requirements for virion assembly and release, we have designed self-assembling protein nanoparticles that are released from cells inside membrane envelopes.

We used a dodecahedral assembly that was generated based upon the trimeric aldolase 1wa3 as basis to design enveloped nanoparticles. Addition of the HIV-1 MA myristoylation signal targeted these assemblies to membranes, and addition of the HIV-1 p6 protein recruited the ESCRT pathway to support efficient release of the nanoparticles within vesicles. Cryo-electron microscopic analyses demonstrated that the protein assembled as designed and that each released vesicle contained multiple nanoparticles. Pseudotyping with the VSV-G envelope protein allowed these vesicles to fuse with new target cells, as assayed by successful packaging and delivery of a β-lactamase-Vpr fusion protein. This overall strategy for creating enveloped protein nanoparticles is very general and we have now demonstrated that different membrane binding sequences, self-assembling proteins and ESCRT recruiting elements can be combined to produce extracellular enveloped protein nanoparticles. These systems will now be used to analyze how different variables affect the efficiency and properties of nanoparticle envelopment and release, and used as a starting point for creating new synthetic delivery systems based on the principles employed by enveloped and non-enveloped viruses.

F33. Virus-like Particles of Immature HIV-1 Assembled on Bacteriophage-derived Templates

Pooja Saxena[1], Li He[2†], Andrey Malyutin[1‡], Siddhartha A. K. Datta[3], Alan Rein[3], Kevin M. Bond[1], Martin F. Jarrold[1], Alessandro Spilotros[4], Dmitri Svergun[4], Trevor Douglas[1] and Bogdan Dragnea[1]

[1]Department of Chemistry, Indiana University, Bloomington, IN 47405, USA; [2]Department of Molecular and Cellular Biochemistry, Indiana University, Bloomington, IN 47405, USA; [3]National Cancer Institute, P.O. Box B, Building 535, Frederick, MD 21702-1201, USA; [4]European Molecular Biology Laboratory-DESY, Notkestrasse 85, Geb. 25a, 22603 Hamburg, Germany; Present addresses: [†]Department of Genetics, Yale School of Medicine, 333 Cedar Street, New Haven, CT 06510; [‡]Columbia University Medical Center, Department of Biochemistry and Molecular Biophysics, 650 W 168th St, Black Building 2-221, New York, NY 10032

Immature human immunodeficiency virus type 1 (HIV-1) particles are pleomorphic, quasi-spherical structures, built from the structural protein, Gag. The architecture of the immature particles is dominated by the hexameric lattice formed by radially arranged Gag molecules. Unlike regular icosahedral viruses, where closure of the hexagonal lattice is mediated by 12 pentameric vertices, in HIV this closure is mediated by the presence of large defects (gaps) in the Gag lattice. The nature and distribution of these defects in the Gag lattice are not well characterized. We have attempted to impose a constraint on lattice curvature by assembling Gag upon modified bacteriophage P22 heads. This template is monodisperse in size and is electron-transparent, enabling the use of cryo-electron microscopy in structural studies. When Gag is forced to assemble under templated constraints, the resulting assemblies are far less polydisperse than any previously described virus-like particles (and, while constructed from the same lattice as natural particles, contains almost no gaps in the lattice). The organization of Gag in these assemblies, and the implications to the natural Gag lattice are discussed. The possible use of these templates to dissect the mechanism in operation in assembly-defective Gag mutants is also considered.

F34. Matrix Mutations Responsible for Retargeting Gag to MVBs also Decrease Matrix's Affinity for $tRNA^{Lys3}$

Christy R. Gaines, Amalia Rivera-Oven, Emre Tkacik, Ally Yang, Alecia Achimovich, Tawa Alabi, and Michael Summers

Howard Hughes Medical Institute at UMBC

The matrix (MA) domain of the HIV-1 Gag polyprotein is responsible for targeting the Gag complex to the plasma membrane for virion assembly. This targeting is mediated through specific interactions between the highly basic patch (HBR) on matrix and phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] on the plasma membrane. Mutations of basic residues in the HBR can lead to retargeting of Gag from the plasma membrane to multivesicular bodies (MVBs). In addition to $PI(4,5)P_2$, MA will specifically bind to some cellular tRNAs, including the $tRNA^{Lys3}$ which is packaged into virions and serves as the primer for reverse transcription. Isothermal titration calorimetry (ITC) was used to investigate MA and $tRNA^{Lys3}$ interactions, and to determine the role basic patch residues play in MA's ability to bind $tRNA^{Lys3}$. Mutations that cause retargeting of Gag to MVBs also resulted in decreased affinity of MA for $tRNA^{Lys3}$. Mutations in K32 resulted in almost complete abolishment of binding, while mutations in K30, K26, and K27 weakened MA-tRNA interactions.

F35. HIV-1 Matrix-31 Membrane Binding Peptide Interacts Differently with Membranes Containing PS vs. PI(4,5)P_2

Lauren O'Neil[1], Kathryn Andenoro[1], Isabella Pagano[1], Laura Carroll[1], Leah Langer[1], Zachary Dell[1], Davina Perera[2], Bradley W. Treece[1], Frank Heinrich[1,3], Mathias Lösche[1,3,4], John F. Nagle[1] and Stephanie Tristram-Nagle[1]

[1]Biological Physics Group, Physics Department, Carnegie Mellon University, Pittsburgh, PA 15213; [2]Biomedical Engineering, Douglass College, Rutgers University, New Brunswick, NJ 08901; [3]National Institute of Standards and Technology Center for Neutron Research, Gaithersburg, MD 20899; [4]Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213

Efficient assembly of HIV-1 at the plasma membrane (PM) of the T-cell specifically requires PI(4,5)P_2. It was previously shown that a highly basic region (HBR) of the matrix protein (MA) on the Gag precursor polyprotein $Pr55^{Gag}$ is required for membrane association. MA is N-terminally myristoylated, which enhances its affinity to membranes. In this work we used X-ray scattering and neutron reflectivity to determine how the physical properties and structure of lipid bilayers respond to the addition of binding domain peptides, either in the myristoylated form (MA_{31}myr) or without the myristoyl group (MA_{31}). Neutron reflectivity measurements showed the peptides predominantly located in the hydrocarbon interior. Diffuse X-ray scattering showed differences in membrane properties upon addition of peptides and the direction of the changes depended on lipid composition. The PI(4,5)P_2-containing bilayers softened, thinned and became less ordered as peptide concentration increased. In contrast, POPS-containing bilayers with equivalent net charge first stiffened, thickened and became more ordered with increasing peptide concentration. As softening the host cell's PM upon contact with the protein lowers the free energy for membrane restructuring, thereby potentially facilitating budding of viral particles, our results suggest that the role of PI(4,5)P_2 in viral assembly goes beyond specific stereochemical membrane binding. These studies reinforce the importance of lipids in virology.

F36. Cholesterol Enhancement of Retroviral Gag Protein-Membrane Interaction: Mechanistic Insights

Milka Doktorova[1], Fred Heberle[2,3], Richard Kingston[4], John Katsaras[3], Gerald Feigenson[5], Harel Weinstein[6], Volker Vogt[5], and Robert Dick[5]

[1]Weill Cornell Medical College, Tri-Institutional Program in Computational Biology and Medicine; [2]Oak Ridge National Laboratory, Biology and Soft Matter Division; [3]University of Tennessee, Bredesen Center for Interdisciplinary Research and Graduate Education; [4]School of Biological Sciences, University of Auckland; [5]Cornell University, Department of Molecular Biology and Genetics; [6]Weill Cornell Medical College, Department of Physiology and Biophysics

Membrane binding of the retroviral structural protein Gag involves the matrix (MA) domain interacting with the inner leaflet of the plasma membrane (PM). Prominent among the interactions is electrostatic attraction between the positively charged MA domain and the negatively charged PM inner leaflet. Previously, we reported that membrane association of reticulocyte extract-generated HIV-1 Gag, as well as purified RSV MA and Gag, are strongly enhanced by physiological membrane fractions of cholesterol. The mechanism underlying this enhancement was unclear. Using small angle neutron scattering (SANS), all-atom molecular dynamics (MD) simulations, membrane surface potential calculations, and protein binding measurements, we addressed the question: How does cholesterol stimulate RSV MA binding to membranes? In SANS experiments with liposomes in the presence or absence of cholesterol, MA binding did not significantly alter bilayer structure, but resulted in a modest lateral redistribution of POPS lipids underneath the bound MA. MD showed small conformational rearrangements in the protein that facilitated electrostatic interactions between MA and the bilayer. Both SANS and MD indicated a large cholesterol-induced reduction in the average lipid area; at a fixed POPS concentration of 30 mol%, this resulted in increased membrane surface charge density. Although in vivo MA-liposome association is highly dependent on lipid composition and ionic strength, the application of continuum electrostatic theory revealed a universal dependence on membrane surface potential, with binding increasing dramatically at a surface potential of -80 to -95 mV. Together, our results conclusively show that cholesterol enhances RSV MA binding by modulating the electrostatic surface potential of the membrane.

F37. Molecular Determinants of Retroviral Gag Membrane Assembly

Frank Heinrich,[1,2] Marilia Barros,[1] Rebecca Eells,[1] Ioannis Karageorgos,[3,4] Hirsh Nanda,[1,2] Robert A. Dick,[5] Volker M. Vogt,[5] Sid A. K. Datta,[6] Alan Rein,[6] and Mathias Lösche[1,2]

[1]Dept. of Physics and Dept. of Biomedical Engineering, Carnegie Mellon University; [2]The NIST Center for Neutron Research; [3]Biomolecular Measurement Division, NIST; [4]Institute for Bioscience and Biotechnology Research (IBBR); [5]Dept. of Molecular Biology and Genetics, Cornell University; [6]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, NIH

The N-terminal matrix domain of retroviral Gag, MA, is the key structural motif that mediates Gag membrane binding which results in the formation of a two-dimensional protein lattice within the protein/membrane shell of budding virions. Several complementing mechanisms are implicated in MA membrane binding: electrostatic interactions between a patch of basic amino acids on MA and anionic lipids, hydrophobic interactions of MA's myristoylated N-terminus with the bilayer, and specific binding to phosphatidylinositolbisphosphate, $PI(4,5)P_2$, in the plasma membrane. We used solid-supported, sparsely-tethered bilayer lipid membranes (stBLMs) to study structural, functional and thermodynamic aspects of Gag association with lipid bilayers and to quantify MA binding to membranes of various compositions as a function of protein concentration. For HIV Gag, which is N-myristoylated, a comparative study of –myrMA and +myrMA shows that lipidation increases MA membrane association significantly, but its contribution to the free energy of binding is much less than the insertion energy of a free myristoyl chain into a membrane. Our results argue against the hypothesis that PIP_2 binding activates myristoyl exposure at the plasma membrane and agree with vesicle (LUV) flotation assays in qualitative, but not in quantitative terms, as we observe membrane binding with micromolar affinities. For RSV Gag, which is not lipidated, we were able to compare membrane binding of the full-length protein with that of MA and find that protein-protein interactions between full-length proteins increase membrane affinity by two orders of magnitude. Cholesterol increases MA membrane binding significantly, both by facilitating membrane insertion of the myristate and protein binding to $PI(4,5)P_2$. Structural investigations with neutron reflection of MA bound to stBLMs show the shallow penetration of the cationic protein surface into the bilayer and determine the orientation of the protein on the membrane surface.

F38. Membrane Charge and Order Influence Membrane Binding of the Retroviral Structural Protein Gag

Yi Wen, Robert A. Dick, Gerald W. Feigenson and Volker M. Vogt

Department of Molecular Biology and Genetics, Cornell University, Ithaca NY 14853

The retroviral Gag polyprotein provides the principal driving force for virus assembly and budding from the plasma membrane (PM). Several principles govern Gag-membrane binding, including electrostatic and hydrophobic interactions, Gag multimerization, and recognition of both lipid head groups and acyl chains. It has been said that retroviruses “bud from rafts”, but this concept remains ill-defined. Published in vitro experiments with giant unilamellar vesicles (GUVs) with two lipid phases do not discriminate between Gag-related proteins recognizing lipid order itself (Lo or Ld phases) or recognizing the phase with the highest negative charge. To better understand how Gag interacts with the PM, we have designed and purified fluorescent electrostatic sensor proteins, such as GFP with a C-terminal tail of 4, 8, or 12 basic residues. The liposome binding of these proteins was compared to that of purified RSV Gag. The anionic lipids PS and $PI(4,5)P_2$ contributed to the recruitment of these polycationic proteins, as expected. Cholesterol also enhanced the binding of the polycationic proteins to membranes with fixed PS concentrations, similar to what we have previously reported for RSV and HIV MA and Gag. Both the electrostatic sensor proteins and RSV Gag, which was fluorescently labeled at its C-terminus at an 11-residue epitope for phospho-pantetheinyl transferase, had similar responses to acyl chain order (Lo or Ld) and head group type. We have used fluorescence resonance energy transfer to create a partial lipid phase diagram to allow the percentage of PS in co-existing Lo and Ld phases to be calculated. On GUVs, the electrostatic sensor proteins bound to the PS-rich phase, independent of lipid order; similar analyses are now being done for Gag. We are developing fluorescence correlation spectroscopy (FCS) as a method to rapidly measure protein-membrane binding constants. We plan to use GUVs with lipid compositions similar to those of the PM inner leaflet to study Gag assembly.

F39. Co-Crystallization of Nucleotides with HIV Protease Reveals a Potential Mechanism for Rate Enhancement in the Presence of RNA

Tiefenbrunn, T[1]., Happer, M.[2], Lin, Y.-C.[2], Elder, J. H.[2], Stout, C. D.[1,*]

*Departments of [1]ICSB, [2]IMS, TSRI, 10550 N. Torrey Pines Rd., La Jolla, CA 92037; *Deceased April 2016*

Recent work by the Swanstrom group demonstrated increased proteolytic activity of HIV protease (PR) in the presence of various RNA molecules (J. Mol. Biol., 2015 Jul 17;427(14):2360-78). To determine if a stable, direct interaction of nucleotides with PR was responsible for this interaction, co-crystallization studies were undertaken with several of the more rate-accelerating RNAs identified in Swanstrom's report and with individual nucleotides, including 3'-adenosine monophosphate (3'-AMP) and 3',5'-adenosine diphosphate (3',5'-ADP). Co-crystals of PR with 3'-AMP and 3',5'-ADP were obtained and diffracted to ~3.5 Å resolution. For several of these structures, the crystal packing is novel and in a space group not previously described for PR out of the 800+ PR structures in the PDB. While the resolution does not allow atomic-level details to be observed, interestingly, the nucleotides appear to be interacting at the dimer interface of PR. Residues at the dimer interface have been reported to alter the relative cleavage rates of sites within GagPol, so interactions at this site could be responsible for the observed activation effects of RNA with PR.

F40. Analyzing the Hydration Structure of HIV-1 Protease using Molecular Dynamics Simulations

Florian Leidner,[1] Janet Paulsen,[1] Nese KurtYilmaz,[1] Yves Muller,[2] and Celia Schiffer[1]

[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, USA; [2]Division of Biotechnology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

HIV-1 protease is essential for viral maturation and one of the main targets of antiretroviral therapy. Under the selective pressure of therapy, drug resistant strains have emerged. Residues in the active site of HIV-1 protease are particularly prone to resistance mutations if their interactions with the inhibitor outweigh interactions with the enzyme substrate. Recently developed protease inhibitors such as darunavir fit well within the substrate consensus volume and minimize these contacts, requiring additional mutations distal from the active site for resistance to occur. These distal mutations influence the active site through networks of intramolecular interactions, reducing inhibitor potency while maintaining enzymatic activity. A plethora of studies aimed to understand the intramolecular rearrangements that lead to resistance, however few have looked beyond the macromolecular surface and considered the role of hydration waters in mediating intramolecular interactions. In this study we utilize molecular dynamics simulations to elucidate the role of hydration in the structural ensemble of HIV-1 protease. We identified local maxima in the solvent density distribution where water–protein interactions significantly perturb solvent dynamics. Water molecules occupying these sites differ significantly from bulk water. Based on their location relative to the protein we assigned each peak to one of three categories: (A) Long lived buried sites in slow exchange with the bulk solvent; (B) Transiently occupied buried sites; (C) Surface site in rapid exchange with the bulk solvent. While type A sites are well resolved in protein crystallography, type B and C sites elude crystallographic approaches due to both their lower electron density and crystal packing effects. Our results resolve and characterize the full structure of HIV-1 protease hydration. We demonstrate how water mediated interactions expand the network of intra- and intermolecular interactions. Future work will address how rearrangements of the enzymes structure due to drug resistance mutations correlate with changes in hydration structure. We expect our results to complement the current models of drug resistance, and expand our understanding of the molecular mechanisms underlying drug resistance to aid in the future development of more robust inhibitors.

F41. Design, Stereoselective Synthesis and Evaluation of HIV-1 Protease Inhibitors Incorporating Novel P2′ Groups

Linah N. Rusere,[1] Akbar Ali,[1] Sook-Kyung Lee,[2] Ronald Swanstrom,[2] and Celia A. Schiffer[1]

[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605, United States; [2]Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, United States

Currently approved HIV-1 protease inhibitors are prone to drug resistance due to the rapid evolution of HIV. Darunavir (DRV) has shown great promise in the treatment of drug resistant HIV, but emerging resistance strains are challenging its efficacy. It is, therefore, necessary to develop new antiviral drugs that retain potency against drug resistant HIV variants. Previously, our lab used the substrate envelope model to design analogs of DRV with variable P1′ and P2′ moieties, which exhibited improved antiviral potencies than DRV against a large panel of drug-resistant HIV strains. Based on these promising leads, we designed two new series of HIV-1 protease inhibitors containing modified P2′ groups to further improve inhibitor potency. The inhibitors that contain the dihydroxyl group at the P2′ exhibited significant antiviral activity against wild-type HIV, although they were relatively less potent than DRV. The corresponding monohydroxyl inhibitors displayed exceptionally high antiviral potencies, two- to five-fold better than DRV. These lead inhibitors are currently being tested for antiviral activity against drug resistant HIV strains.

F42. Long-Range Structural Perturbation upon Active-Site Inhibitor Interaction on HIV-1 Protease

Shahid N Khan, John D Persons, Michel T Guerrero, and Rieko Ishima

Department of Structural Biology, University of Pittsburgh School of Medicine

HIV-1 protease (HIV-1 PR), a homodimer with two 10 kDa subunits, is a key enzyme for viral replication in HIV. Drugs that inhibit the PR activity have been used for AIDS therapeutics. However, emergence of drug resistance of HIV-1 PR against inhibitors is a therapeutic limit. Schiffer's group [1] developed a series of highly potent PR inhibitors that were derived from an FDA approved anti-protease drug, darunavir (DRV). Although PR crystal structures bound to these inhibitors are all very similar to that of DRV-bound form, characteristics of inhibitor-binding affinities to WT and resistant mutants differ among the inhibitors. Here, we investigated structural and conformational impact of these newly developed potent inhibitors against wild-type HIV-1 PR at atomic level using NMR, with a hypothesis that subtle conformational changes in solution may explain such different profiles in inhibition and resistance. Comparison of amide chemical shift and relaxation data analysis of PR bound to these inhibitors with that to DRV indicates significant long-range effect, over 16 Å, that was not detected previously. Analysis of chemical shifts of PR upon binding to a series of inhibitors indicates that the long-range effect is asymmetric between the two subunits of PR, and reflects differences in pharmacophore among the inhibitors. These long-range effects may explain why the remote mutations occur in conferring drug resistance. Project supported by NIH P01 GM109767

1. Nalam et al, Chem & Biol, 20, 1116-24 (2013)

F43. Exploring Surface Sites on HIV Protease as Targets for Inhibitors: From Computation to Biological Activity

Tiefenbrunn, T.[1], Forli, S.[1], Happer, M.[2], Gonzalez, A[3]., Baksh, M. M.[4], Chang, M. W.[5], Tsai, Y.-S.[3], Lin, Y.-C.[2], Perryman, A. L.[1], Rhee, J.-K.[4], De Vera, I.[6], Kojetin, D.[6], Torbett, B.E.[5], Olson, A. J.[1], Soltis, M.[3], Elder, J. H.[2], Finn, M. G.[4], and Stout, C. D.[1,*]

*Departments of [1]ICSB, [2]IMS, [5]MEM, TSRI, 10550 N. Torrey Pines Rd., La Jolla, CA 92037; [3]SSRL, SLAC, 2575 Sand Hill Road MS 99, Menlo Park, CA 95124; [4]School of Chemistry & Biochemistry, Georgia Tech, 901 Atlantic Drive, Atlanta, GA 30332; [6]Department of Molecular Therapeutics, TSRI Florida, 130 Scripps Way, Jupiter, FL 33458; *Deceased April 2016*

A crystallographic fragment-based screen against HIV protease (PR) identified two surface sites, the flap site and the exosite, that are potentially important for protein-protein interactions with Gag and are alternatives to the active site for drug design. Co-crystals of HIV PR with a fragment library revealed several small fragment molecules binding in the exosite. Based on these fragment hits, computational studies in the Olson group identified 1-(4-methylphenyl)sulfonyl-3-(1,3-thiazol-2-yl)urea (C6) as a potential exosite binder. C6 binds to PR as indicated by DSF and BSI assays, is weakly inhibitory in an enzymatic cleavage assay (56% inhibition of protease at 1 mM) and has an additive effect when combined with the weak active site inhibitor pepstatin. C6 has been co-crystallized in two crystal forms of TL-3:PR, conserving interactions observed for other fragments. The observed binding mode of C6 involves a portion of the molecule being solvent exposed, suggesting modes for fragment refinement to more closely interact within the exosite pocket of PR.

Based on these results, a new series of compounds was developed and tested for the inhibition of protease. Several larger fragment hits were obtained and their binding to PR was measured by NMR, both with and without active site inhibition by the high-affinity active site inhibitor TL-3. The compounds SF4 and SF9 both appear to interact in the exosite of PR and demonstrate an additive effect of inhibition in combination with the weak active site inhibitor pepstatin. Co-crystals of PR and SF9 have been obtained, but the crystals did not diffract beyond 8Å resolution.

F44. Interdependence of Inhibitor Recognition in HIV-1 Protease Sub-Sites

Janet Paulsen, Florian Leidner, Akbar Ali, and Celia Schiffer

Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, USA

Our lab has developed the substrate envelope hypothesis to link structure to mechanisms of resistance. This hypothesis asserts that inhibitors designed to best fit the recognition site volume of the natural substrates will be less susceptible to resistance. Darunavir (DRV), the most potent and least susceptible to drug resistance of all FDA approved inhibitors targeting HIV-1 protease, fits well with the substrate envelope. Substrate recognition for HIV-1 protease is based upon the shape of the substrate envelope; however, the dynamic interplay of the sub-sites determines this shape. A series of DRV analogs has been designed to use as probes for investigating the dependence of sub-sites. Using this series of DRV analogs rigorous molecular dynamics simulations are examined to understand how the ligand diversity at different sub-sites influences important chemical properties and energetics. Elucidation of this “push-pull” between the sub-sites will aid in future design of more robust inhibitors that are less prone to resistance.

F45. Improving Inhibitor Design to Counter Drug Resistance: Lessons from HIV-1 and HCV Protease Inhibitors

Nese Kurt Yilmaz[1], Ronald Swanstrom[2] and Celia A. Schiffer[1]

[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605, USA; [2]Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA

Drug resistance is a major burden in a variety of human diseases, undermining therapy outcomes and necessitating novel approaches to drug design. Quickly evolving drug targets, such as HIV-1 and hepatitis C virus (HCV) protease, rapidly select for mutations that weaken inhibitor binding while still allowing biological function and viral propagation. Resistance to viral protease inhibitors has been the target of extensive investigation, revealing the structural and molecular mechanisms underlying resistance. These mechanisms allowed us to propose strategies to improve the design of viral protease inhibitors to counter resistance, mainly by exploiting the essential biological function and leveraging evolutionary constraints. This entails design not just against the wild-type target but considering all potential mutant variants that can exist and still carry out the protease's biological function. Combining strategies to minimize vulnerability to resistance while enhancing potency can lead to inhibitors that are more robust against resistance. Regardless of whether 'resistance-proof' inhibitors are achievable or not, lessons from HIV-1 and HCV proteases can be incorporated into structure-based drug design not only for viral proteases but for other quickly evolving drug targets as well.

F46. Mapping The Fitness Landscapes of Drug-Resistance in HIV Protease

Jeffrey I Boucher[1], Troy W Whitfield[1], Sook-Kyung Lee[3,4], Ronald Swanstrom[2,3,4], Celia A Schiffer[1], and Daniel NA Bolon[1]

[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester MA 01605, USA; [2]Department of Microbiology and Immunology, [3]Department of Biochemistry and Biophysics, and the [4]UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

The evolution of drug resistance is a pressing issue in human diseases ranging from bacterial and viral infections to cancer. Chemical intervention applies the selective pressure necessary for the fixation of resistant variants. Current efforts to determine the resistance potential of drug targets rely on stochastic experiments, such as adaptive laboratory evolution and the analyses of clinical isolates. Systematic mutational scanning provides a promising alternative by comprehensively mapping the resistance landscape of a protein to actively inform preclinical drug design. HIV-1 protease is an ideal model system to evaluate the scanning approach due to the theoretical potential for circulating viral populations to have fully explored all possible resistance mutations and the wealth of sequenced resistant isolates. To determine the local fitness landscape of HIV protease, we have generated a library of all single amino acid substitutions and evaluated each mutation's effect on resistance to clinically approved protease inhibitors. Since highly resistant clinical isolates feature multiple mutations, we have also analyzed a combinatorial library of known resistance mutations in order to identify the key epistatic interactions that shape the evolutionary pathways leading to high resistance. The results of these comprehensive screens will be compared to the database of resistant clinical isolates to critically evaluate the potential of systematic mutational scanning to map resistance potential and inform preclinical drug design.

F47. The HIV-1 Late Domain-2 S40A Polymorphism in Antiretroviral Exposed Individuals Influences Protease Inhibitor Susceptibility

Susan M Watanabe[1], Viviana Simon[2], Brittney R Kemp[3], Satoshi Machihara[3], Kimdar Sherefa Kemal[4], Binshan Shi[5], Brian Foley[6], Barbara Weiser[7,8], Harold Burger[7,8], Kathryn Anastos[4], Chaoping Chen[3], and Carol A Carter[1]

[1]Stony Brook University, Dept. of Molecular Genetics & Microbiology, Stony Brook, NY; [2]Icahn School of Medicine at Mount Sinai, Dept. of Microbiology, Global Health and Emerging Pathogens Institute, New York, NY; [3]Colorado State University, Dept. of Biochemistry and Molecular Biology, Denver, CO; [4]Albert Einstein College of Medicine, Dept. of Medicine, New York, NY; [5]Albany College of Pharmacy and Health Sciences, Dept. of Health Sciences, Albany, NY; [6]Los Alamos National Laboratory, Theoretical Biology and Biophysics Dept., Los Alamos, NM; [7]University of California Davis, Dept. of Medicine, Sacramento, CA; [8]Sacramento VA Medical Center, Dept. of Medicine, Sacramento, CA

The p6 region of the HIV-1 structural precursor polyprotein, Gag, contains two motifs, P_7TAP_{10} and $L_{35}YPLXSL_{41}$, designated as late (L) domain-1 and -2, *respectively*. These motifs bind the ESCRT-I factor Tsg101 and the ESCRT adaptor Alix, *respectively*, and are critical for efficient budding of virus particles from the plasma membrane. L domain-2 is thought to be functionally redundant to PTAP. To identify possible other functions of L domain-2, we examined this motif in dominant viruses that emerged in a group of 14 women who had detectable levels of HIV-1 in both plasma and genital tract despite a history of current or previous antiretroviral therapy. Remarkably, variants possessing mutations or rare polymorphisms in the highly conserved L domain-2 were identified in six of these women. A mutation in a conserved residue (S40A) that does not reduce Gag interaction with Alix and therefore did not reduce budding efficiency was further investigated. This mutation causes a simultaneous change in the Pol reading frame but has no significant deficiency in Gag processing or virion maturation. When introduced into the HIV-1 NL4-3 strain genome, S40A altered sensitivity to the protease (PR) inhibitor indinavir (IDV). When introduced into a model PR precursor, S40A reduced production of mature PR and accumulated two extended forms of PR; one was fairly stable in the presence of IDV and the other was effective in *trans* processing with micromolar IDV. Our results indicate that L domain-2, considered redundant *in vitro*, can undergo mutations in vivo that significantly alter PR function. These may contribute to high levels of replication in both the absence and presence of PR inhibitors.

This work is supported by NIH NIAID R01 AI068463, NIGMS R01 GM111028 and ARRA supplemental funding awards to CAC; NIH NIAID R21A1080351 and R03AI108392 to CC; NIH U3U 01AI35004 award to KA and NIH NIAID R01AI064001 to VS.

F48. The Contribution of Mutations Outside of the Protease Appeared During DRV Selection in Conferring Resistance to Highly Potent Protease Inhibitors

Sook-Kyung Lee[1], Ean Spielvogel[1], Shuntai Zhou[1], Jesse Crayle[1], Celia Schiffer[2], and Ronald Swanstrom[1]

[1]Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina, Chapel Hill, NC; [2]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts, Worcester, MA

We previously carried out in vitro selection of HIV-1 growing in CEM×174 cells for resistance to Darunavir (DRV) using a mixture of 26 variants, each containing a single PI-resistance mutation as the starting virus. Bulk sequencing analysis using cell pellet DNA collected from the last passage, p42, revealed additional mutations accumulated outside of the protease region (in Gag, RT, IN, and the gp41 C-Terminal Tail region) in addition to resistance mutations within the protease coding domain. Most of these mutations reside beyond the protease cleavage sites. The role of mutations outside of the protease (and its processing sites) as contributors to resistance is poorly understood. To examine the contribution of mutations outside of the protease in conferring resistance to highly potent protease inhibitors, we are in the process of generating pNL-CH molecular clones containing the separate coding domains for Gag, PR, RT, IN, Gag-PR, PR-RT, or PR-IN as found in the final passage of the DRV culture. To amplify *gag-pro-pol* region, first we carried out single genome amplification (SGA). Based on bulk sequence analysis of SGA PCR products, we chose a single DNA template that includes all of the consensus mutations seen from multiple DNA templates. Currently, Gag, PR, IN, Gag-PR, and PR-IN regions containing mutations selected by DRV have been cloned into the background of pNL-CH and we are in the process of assessing the effects of mutations outside of the protease region on viral infectivity and PI resistance. In addition, cloning of RT and PR-RT regions containing mutations selected by DRV into pNL-CH is underway.

F49. Effects of Natural Polymorphisms of non-B HIV-1 Protease on Protein Conformations

Trang Tran[1], Zhanglong Liu[1], Ben M. Dunn[2], and Gail E. Fanucci[1]

[1]Department of Chemistry, University of Florida, Gainesville, FL 32611; [2]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610

HIV-1 protease (PR) is essential to the maturation of HIV-1 virus as it post-translationally cleaves the viral polyproteins gag and gag-pol. Inhibition of HIV-1 PR lead to non-infectious immature virus. This makes HIV-1 PR a drug target for HIV infection. The flaps of HIV-1 PR play an important role in its catalytic activity as they control access of substrate as well as inhibitor to the catalytic pocket of the protease. The flaps of HIV-1 PR adopt different conformations: closed, semi-open, wide-open, and curled-open. Our previous study suggests natural polymorphisms can affect conformational ensembles of the protease, hence protein dynamics. In this study, electron paramagnetic resonance (EPR) and double electron-electron resonance (DEER) are utilized to study how natural polymorphisms affect protein conformational ensembles in non-B HIV-1 PR variants including subtypes H, F, and D, and CRFs AC, BF, and AG.

F50. Inference of Epistatic Effects and the Development of Drug Resistance in HIV-1 Protease

William F. Flynn[1,2], Allan Haldane[2], and Ronald M. Levy[2]

[1]Department of Physics and Astronomy, Rutgers University, Piscataway, NJ; [2]Center for Biophysics and Computational Biology, Department of Chemistry, Temple University, Philadelphia, PA

Understanding the complex mutation patterns that give rise to drug resistant viral strains provides a foundation for developing more effective treatment strategies for HIV/AIDS due to rapid evolution under intense selective pressure. Multiple sequence alignments of drug-experienced HIV-1 protease sequences contain networks of strong pair correlations which can be used to build a (Potts) Hamiltonian model of these mutation patterns. Using this Hamiltonian model we translate HIV protease sequence covariation data into quantitative predictions for the stability and fitness of individual proteins containing therapy-associated mutations which we compare to previously performed *in vitro* measurements of protein stability and viral infectivity. We show that the penalty for acquiring primary resistance mutations depends on the epistatic interactions with the sequence background and, although often destabilizing in a wildtype background, primary mutations are frequently stabilizing in the context of mutation patterns which arise in response to drug therapy. Anticipating epistatic effects has implications for the design of future protease inhibitor therapies.

F51. Altering the Conformational Landscape as a Mechanism for Evolution in HIV-1 Protease

Zhanglong Liu, Lingna Hu, Linh Pham, Trang T. Tran, Xi Huang, Ben M. Dunn, and Gail E. Fanucci

Department of Chemistry, University of Florida, Gainesville, FL 32611

Natural polymorphisms that occur in non-B clade HIV-1 protease (PR) are oftentimes secondary mutations associated with drug-pressure selected evolution in clade B multi-drug resistance. We have hypothesized a conformational selection model to explain the impact that natural polymorphisms and drug-pressure selected mutations have on protease kinetics and interaction with inhibitors. Here we summarize experimental spectroscopic work derived from EPR based distance measurements compiled for a series of PR constructs that have developed drug resistance (some specific to Darunavir or Nelfinavir or from clinical isolates with mixtures of PIs) as well as select natural polymorphisms. Results from new investigations uphold our earlier hypothesis that mutations combine to stabilize open-like states that are more dynamic than the semi-open conformation and reveal a forth conformation we describe as a curled-open state. Results also demonstrate a fine balance of stability of the conformational ensemble in that the semi-open conformation needs to dominate at least ~60% of the population for efficient enzymatic turnover. Hence, mutations combine to flip-flop the relative stability of the closed-like to open-like states. Potentially indicating open-like structures as targets for next generation inhibitors.

F52. Selection to Confirm Novel Resistance Pathways to Potent New UMASS HIV-1 Protease Inhibitor

Ean Spielvogel[1], Sook-Kyung Lee[1], Shuntai Zhou[1], J Paulsen[2], Celia Schiffer[2], and Ronald Swanstrom[1]

[1]Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina, Chapel Hill, NC; [2]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts, Worcester, MA

From the previous selection for resistance to UMASS 1-10 including DRV and a no-inhibitor control, we observed three unique resistance patterns associated with specific structures in the inhibitors. To confirm these results, we have been carrying out selection in CEMX174 cells using wild type virus, NL-CH derived from NL4-3, as the starting virus. We also used 1 nM as an initial inhibitor concentration based on EC50 values of UMASS PIs which ranged from 2 nM to 9 nM. Currently, the starting NL-CH virus has undergone 26 to 50 passages where drug concentrations range from 35 nM to 5000 nM. We observed that the inhibitors fall into two groups depending on the rate of increasing drug concentration that still allows robust virus replication in the culture: rapid (UMASS-1, 4, 6, 7, 8, 9, and 10) and slow (UMASS-2, 3, 5, and DRV). Inhibition of virus replication was apparent as the drug level reached the EC50. We performed deep sequencing using viral RNA from the passage at a certain drug concentration. Measurements took place in ½ log increments based on our original EC50 data. Currently, measurements have been taken at five time points of 10, 30, 100, 300, 1000, and 3000 nM. We detected up to 10 major mutations in the protease region, including V82I (except UMASS-6), A71V (except UMASS-1, 7), M46I (except UMASS-4), and I50V (except UMASS-1 and 4). We also detected presence of transitionary minor resistance mutations even with low selective pressure.

F53. Mechanistic Characterization of Inhibitor Resistance in HIV-1 Protease via Machine Learning and Atomistic Simulation

Debra A. Ragland[1], Troy W. Whitfield[2,3], Nese Kurt-Yilmaz[1], Konstantin Zeldovich[1,3] and Celia A. Schiffer[1]

[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester MA 01655; [2]Department of Medicine, University of Massachusetts Medical School, Worcester MA 01655; [3]Program in Bioinformatics and Integrative Biology, University of Massachusetts Medical School, Worcester MA 01655

Darunavir is an HIV-1 protease inhibitor with high binding affinity that can be effective against strains where resistance to other inhibitors, such as saquinavir, has developed. Despite this effectiveness and the associated delay in the onset of protease resistance to darunavir inhibition, however, resistance has been observed in the presence of multiple simultaneous mutations. In order to gain mechanistic insight on the role that specific mutations play in this resistance, molecular dynamics simulations were carried out on15 selected HIV-1 protease variants, chosen to include wild-type controls, along with strains that are resistant to darunavir *in vivo* and/or *in vitro*. The resulting high-dimensional space of thermodynamic observables among this set of sequence variants can be reduced to interpretable variables using machine learning.

F54. The Race Against Protease Activation Defines the Role of ESCRTs in HIV Budding

Mourad Bendjennat[1] and Saveez Saffarian[1,2,3,#]

[1]Department of Physics and Astronomy, University of Utah, Salt Lake City, Utah, USA; [2]Center for Cell and Genome Science, University of Utah, Salt Lake City, Utah, USA; [3]Department of Biology, University of Utah, Salt Lake City, Utah, USA; [#]Saffarian@physics.utah.edu

HIV virions assemble on the plasma membrane and bud out of infected cells using interactions with endosomal sorting complexes required for transport (ESCRTs). HIV protease activation is essential for maturation and infectivity of progeny virions, however, the precise timing of protease activation and its relationship to budding has not been well defined. We show that compromised interactions with ESCRTs result in delayed budding of virions from host cells. Specifically, we show that Gag mutants with compromised interactions with ALIX and Tsg101, two early ESCRT factors, have an average budding delay of ~75 minutes and ~10 hours, respectively. Virions with inactive proteases incorporated the full Gag-Pol and had ~60 minutes delay in budding. We demonstrate that during budding delay, activated proteases release critical HIV enzymes back to host cytosol leading to production of non-infectious progeny virions. To explain the molecular mechanism of the observed budding delay, we modulated the Pol size artificially and show that virion release delays are size-dependent and also show size-dependency in requirements for Tsg101 and ALIX. We highlight the sensitivity of HIV to budding "on-time" and suggest that budding delay is a potent mechanism for inhibition of infectious retroviral release.

T55. Resistance Pathways for Potent and Broadly Active HIV-1 Maturation Inhibitors; Insights into Gag Structure During Assembly and Maturation

Emiko Urano[1], Sherimay D. Ablan[1], Justin Kaplan[1], Nishani Kuruppu[1], Juan Fontana[2], Alasdair C. Steven[2], Mingzhang Wang[3], Caitlin Quinn[3], Tatyana Polenova[3], David E. Martin[4], T. J. Nitz[4], Carl T. Wild[4], and Eric O. Freed[1]

[1]HIV Dynamics and Replication Program, NCI, Frederick, MD; [2]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE; [3]NIAMS, NIH, Bethesda, MD; [4]DFH Pharma, Inc., Gaithersburg, MD

A betulinic acid-based compound, bevirimat (BVM), the first-in-class HIV-1 maturation inhibitor (MI), acts by blocking a late step of Gag processing, capsid-spacer peptide 1 (CA-SP1) cleavage. A second compound, PF-46396 (PF96), was found to have a similar mode of action despite being structurally distinct from BVM. Although in clinical trials BVM reduced viral loads in HIV-1-infected patients, single-amino-acid polymorphisms in the SP1 region resulted in reduced susceptibility to the compound. To overcome this problem, we developed "second-generation" MIs based on the BVM scaffold. We identified a set of BVM analogs that show potent and broad antiviral activity.

To understand the target and mechanism of action of MIs, we selected for viral resistance. BVM resistance mapped to residues surrounding the CA-SP1 cleavage site, whereas PF96-resistance mutations clustered at the cleavage site and far upstream in CA, including within the major homology region (MHR). Interestingly, a group of these MHR mutants were profoundly PF96-dependent. Propagation of the MHR mutants led to the selection of a second-site compensatory change at SP1 residue 8 (SP1-T8I). Moreover, on its own, the SP1-T8I mutation induced a high level of CA-SP1 accumulation. Structural analysis by cryo-electron tomography demonstrated that both MI treatment and the SP1-T8I mutation stabilize the immature Gag lattice. Similar results were obtained in magic angle spinning NMR, in which SP1-T8I stabilized the SP1 structure and reduced the internal dynamics of the SP1 peptide in tubular assemblies of CA-SP1. These results indicate that SP1-T8I phenocopies the effect of MI binding. In addition, in the NMR experiments, SP1-T8I also induced conformational changes throughout CA, including within the MHR.

Selection experiments with second-generation BVM analogs identified the mutation CA-P157A, located in the MHR. CA-P157A was replication competent and resistant to not only BVM and the second-generation BVM analogs but also to PF96. Pulse-chase data demonstrate that CA-SP1 processing kinetics for CA-P157A are similar to those of the WT. Analysis of the HIV-1 database reveals that Pro157 of CA is conserved in ~99.95% of available sequences, suggesting a high genetic barrier to resistance. The characterization of resistant mutants provides novel insights into the structure of the MI-binding site and the role of SP1 and the CA MHR in virus assembly and viral maturation. We propose a model whereby MIs act in part by stabilizing the CA-SP1 region in the immature Gag lattice; resistance mutations either reverse this stabilizing effect or prevent compound binding.

F56. Dynamic Regulation of HIV-1 Capsid Maturation by Integrated Magic Angle Spinning NMR and Molecular Dynamics Simulations

Mingzhang Wang[1,2], Caitlin M. Quinn[1,2], Juan R. Perilla[4], Huilan Zhang[1,2], Guangjin Hou[1], Rupal Gupta[1,2], Sherimay Ablan[5], Emiko Urano[5], Jinwoo Ahn[2,3], In-Ja Byeon[2,3], Christopher Aiken[2,6], Klaus Schulten[4], Angela M. Gronenborn[2,3], Eric O. Freed[5], and Tatyana Polenova [1,2]

[1]University of Delaware, Department of Chemistry and Biochemistry, Newark, DE; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 5th Ave., Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA; [4]University of Illinois, Theoretical and Computational Biophysics Group, Urbana, IL; [5]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Frederick, MD; [6]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN

HIV-1 viral maturation is an attractive target for therapeutic intervention. The final step in the Gag processing cascade is the cleavage of spacer peptide 1 (SP1) from the C-terminal domain of capsid (CA). Upon cleavage, the capsid protein condenses to form the capsid shell and results in an infectious particle. We demonstrate that dynamic allostery[1] plays a crucial role in the HIV-1 capsid maturation, by examining the conformation and dynamics of CA and the SP1 peptide in the assemblies of wild type (WT) CA-SP1 and those containing a T8I mutation in the SP1. The T8I mutation abolishes the CA-SP1 cleavage by stabilizing the immature CA-SP1 lattice and thus phenocopies the maturation inhibitor Bevirimat and PF46396[2]. To examine quantitatively, at atomic resolution, the structure and dynamics of HIV-1 CA-SP1 assemblies of WT and T8I mutant in various time scales, we have integrated magic angle spinning (MAS) NMR spectroscopy and molecular dynamics (MD) simulations. The MD simulations of WT CA-SP1 conducted to 30 μs reveal that the SP1 tail is in a helix - coil dynamic equilibrium. This result is consistent with our previous MAS NMR studies, where we have discovered that SP1 is dynamic and adopts a predominantly random coil conformation in tubular assemblies of WT CA-SP1 of HXB2 and NL4-3 sequence variants[3]. The average chemical shifts calculated by MD trajectory show remarkable quantitative agreement with the experimental shifts. In contrast, we find that in the tubular assemblies of CA-SP1 T8I mutant, the SP1 peptide is significantly stabilized with much greater propensity of helical structure. Furthermore, the presence of the T8I mutation induces conformational and dynamics perturbations not only at or in the vicinity of the SP1 peptide but also throughout the entire CA protein, and notably, in functionally important regions, such as CypA loop and MHR. More broadly, our work establishes that by integrating MAS NMR and MD simulations quantitative information can be gained with atomic resolution on dynamically disordered regions of HIV-1 capsid and maturation intermediate assemblies that are invisible by cryo-EM and X-ray diffraction methods. This work was supported by the National Institute of Health (P50GM082251) and is a contribution from the Pittsburgh Center for HIV Protein Interaction. We acknowledge the support of the NSF CHE0959496 grant for acquisition of the 850 MHz NMR spectrometer and of the NIH P30GM110758-01, P30GM103519 grants for the support of core instrumentation infrastructure at the University of Delaware.

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F57. Identification of a Novel Element in HIV-1 CA Critical for Assembly and Maturation

Mariia Novikova[1], Muthukumar Balasubramaniam[1], Sagar Kudchodkar[1], Ferri Soheilian[2], Anna T Gres[3], Karen A Kirby[3], Juan Fontana[4], Alasdair C. Steven[4], Stefan G Sarafianos[3], and Eric O Freed[1]

[1]Virus-Cell Interaction Section, HIV Dynamics and Replication Program, NCI-Frederick, Frederick, MD; [2]Electron Microscope Laboratory, Cancer Research Technology Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD; [3]University of Missouri, C.S. Bond Life Sciences Center, Columbia, MO; [4]Laboratory of Structural Biology Research, National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, MD

Formation of the immature Gag lattice during HIV-1 assembly is driven by interactions between capsid (CA) domains. In this study, we investigated the function of a highly conserved Pro-Pro-Ile-Pro (PPIP) motif (CA residues 122-125) in HIV-1 assembly and maturation. This motif is part of the loop connecting CA helices 6 and 7 (H6-H7 loop) and is at the trimer interface of the immature Gag lattice. We performed alanine-scanning mutagenesis of this motif and showed that mutations P122A and I124A significantly impaired virus release, particle infectivity, and replication in T-cell lines. In addition, using electron microscopy and cryo-electron tomography techniques, we demonstrated that these two mutants displayed obvious defects in formation of the Gag lattice in both PR-containing and PR-deficient virions. In contrast, mutations P123A and P125A were reasonably well tolerated. To better understand the role of the PPIP motif in CA structure and function, we selected for compensatory mutations that rescue the functional defects caused by the P122A and I124A substitutions. Two mutations, V11I and T58A, when present together, rescue the defects imposed by both P122A and I124A such that V11I/T58A/P122A and V11I/T58A/I124A triple mutants are assembly competent, infectious, and replicate with near-WT kinetics. One of the mutants, T58S/T107I/P122A, displayed nearly WT levels of infectivity and WT particle morphology; however, this mutant replicated with a significant delay in Jurkat cells. We showed that this mutant virus is hypersensitive to the CA effector PF74. Crystallographic analysis of T58S/T107I/P122A CA revealed that P122A repositioned the H6-H7 loop and subtly changed residues 92-96 of the proximal CypA-binding loop. T58S rearranged L20 and M39 from neighboring CAs, thus affecting intersubunit interactions. Finally, PF74 susceptibility was likely affected by changes in the PF74 binding pocket, directly through T107I and indirectly through the proximal T58S mutation. Hence, the H6-H7 loop of the HIV-1 CA domain is a new structural element important for contacts in the immature Gag and mature capsid lattices.

F58. Crystal Structure of an HIV Assembly and Maturation Switch

Jonathan Wagner, Kaneil Zadrozny, Jakub Chrustowicz, Michael Purdy, Mark Yeager, Barbie Ganser-Pornillos, and Owen Pornillos

Department of Molecular Physiology and Biological Physics, University of Virginia School of Medicine

Virus assembly and maturation proceeds through the programmed operation of molecular switches, which trigger both local and global structural rearrangements to produce infectious particles. HIV-1 contains an assembly and maturation switch that spans the C-terminal domain (CTD) of the capsid (CA) region and the first spacer peptide (SP1) of the precursor structural protein, Gag. The crystal structure of the CTD-SP1 Gag fragment is a goblet-shaped hexamer in which the cup comprises the CTD and an ensuing type II β-turn and the stem comprises a 6-helix bundle. The β-turn is critical for immature virus assembly and the 6-helix bundle regulates proteolysis during maturation. This bipartite character explains why the SP1 spacer is a critical element of HIV-1 Gag but is not a universal property of retroviruses. Our results also indicate that HIV-1 maturation inhibitors prevent unfolding of the CA-SP1 junction and thereby deny access of the viral protease to its substrate.

F59. Structural Analysis of CA-SP1-NC Assemblies by Magic Angle Spinning NMR

Ryan W. Russell[1,2], Christopher L. Suiter[1,2], Guangjin Hou[1,2], Caitlin M. Quinn[1,2], Angela M. Gronenborn[2,3], Mingzhang Wang[1,2], Manman Lu[1,2], Jinwoo Ahn[2,3], Sherimay D. Ablan[4], Emiko Urano[4], Eric O. Freed[4], and Tatyana Polenova[1,2]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 5th Ave., Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA; [4]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Frederick, MD

The maturation of HIV-1 virus is a critical process in the viral lifecycle necessary for the formation to infectious virions. Maturation outcomes arise through the proteolytic cleavage cascade of Gag polyprotein and results in Gag lattice remodeling. Gag is comprised of matrix (MA), capsid (CA), nucleocapsid (NC), and p6 domains as well as spacer peptides 1 and 2 (SP1 and SP2). The final maturation step is the cleavage of a 14-residue spacer peptide 1, SP1, leading to the formation of a conical capsid comprised of ~1500 copies of a 231-residue CA capsid protein. To understand the structure of the immature Gag lattice, we have studied assemblies of the 300-residue CA-SP1-NC. We have employed multidimensional magic angle spinning (MAS) NMR spectroscopy, to obtain atomic-resolution information on CA-SP1-NC structure. We have assigned the carbon and nitrogen chemical shifts, by both manual and automated procedures, the latter through the FLYA[3] program. From the assigned chemical shifts, we have predicted the secondary structure of CA-SP1-NC in the assembled state, with TALOS-N.[4] The results indicate that (1) the CA domain is folded and its secondary structure is the same as in mature capsids; (2) the NC domain is mostly α-helical, consistent with prior solution NMR studies[5,6] in unassembled NC protein and low-resolution cryo-EM of immature virions[7]; and (3) β-hairpin is not present in the N-terminal domain of CA in the context of CA-SP1-NC. This investigation sets the stage for 3D structure determination of CA-SP1-NC assemblies at atomic resolution by MAS NMR. This work was supported by the National Institute of Health (P50GM082251) and is a contribution from the Pittsburgh Center for HIV Protein Interaction. We acknowledge the support of the NSF CHE0959496 grant for acquisition of the 850 MHz NMR spectrometer and of the NIH P30GM110758-01, P30GM103519 grants for the support of core instrumentation infrastructure at the University of Delaware.

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F60. Dynamic Characterization of The Spacer Peptide 1 (SP1) in Immature HIV-1 Capsid Protein Assemblies

Juan R Perilla[1], Boon Chong Goh[1], Mingzhang Wang[2], Caitlinn Quinn[2], Manman Lu[2], Tatyana Polenova[2], Klaus Schulten[1]

[1]University of Illinois at Urbana-Champaign, Physics and Beckman Institute, Urbana, IL; [2]University of Delaware, Chemistry, Newark, DE

Recent cryo-electron tomography studies of HIV-1, RSV, and M-PMV - have provided comparative analyses of the organization of the Gag lattice and its domains at resolutions that now exceed sub-nanometer detail. In particular, for HIV-1, the region corresponding to the spacer peptide 1 (SP1) has been observed in cryo-electron tomograms. Nonetheless, despite the sub-nanometer resolution of the HIV-1 density maps (8-9 Å), the density corresponding to the SP1 subdomain is not well resolved. Furthermore, MAS-NMR measurements showed that the SP1 subdomain exhibits random coil behavior and is dynamically disordered in CA-SP1 arrangements. Despite all the valuable information describing the interactions of Gag proteins in a lattice environment, not a single technique has been able to determine the atomic structure and dynamics of the oligomerized SP-NC subdomain of HIV-1.

By combining state-of-the-art solid state NMR and over 100s of all-atom molecular dynamics (MD) simulations we derived structural models of the hexameric SP1 subdomain. Our SP1 models include structural restraints that mimic the native Gag lattice observed in authentic HIV-1 virions. Classification of the resulting SP1 conformations was performed according to the difference between predicted and measured MAS-NMR observables. Finally, we reconstructed the kinetic landscape sampled by SP1 in our simulations thus identifying the most probable conformations as well as their transition rates.

F61. Segmental Labeling of HIV-1 Capsid Protein for Solid State NMR Spectroscopy

Sebanti Gupta and Robert Tycko

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney diseases, National Institutes of Health, MD 20892, USA

The mature capsid of human immunodeficiency virus type 1 (HIV-1) encloses the viral genome and ensures the infectivity of the virion in a new host cell. The capsid shell is exclusively made up of 1500 copies of the capsid protein (CA). CA comprises independently folded N-terminal and C-terminal domains (NTD and CTD), separated by a short linker. *In vitro*, full-length CA spontaneously assembles into tubes at high ionic strength, which exhibits structural resemblance to the capsid core in mature HIV-1. Various biophysical techniques have been used to gain structural insight of the mature HIV-1 capsid, but several aspects of this supramolecular structure are yet to be resolved. Solid state NMR spectroscopy is a powerful tool for the characterization and high resolution structure determination of supramolecular complexes. However, successful assignments of NMR resonances in large protein assemblies are often hindered by spectral complexity, resonance overlap, and difficulties in differentiating intra- and intermolecular contacts. This situation could be improved by segmental isotopic labeling of the sample, which would facilitate the sequence specific resonance assignment. Accordingly we have developed a novel split intein based method for preparation of segmentally labeled HIV-1 CA. Subsequently recorded 2D and 3D solid state NMR spectra of segmentally labeled CA tubes reveal significantly reduced spectral overlap when compared with similar spectra of fully labeled CA tubes.

F62. Crystal Structures of, P38A, P38A/T216I, E45A and E45A/R132T HIV-1 Capsid Proteins Highlight the Plasticity of HIV-1 Capsid

Anna T. Gres[1,2], Dandan Liu[1,3], Karen A. Kirby[1,3], Qiongying Yang[1,3], Juan R. Perilla[4], Klaus Schulten[4], Jiong Shi[5,6], Christopher Aiken[5], John J. Tanner[2,6], Xiaofeng Fu[7], Peijun Zhang[7], and Stefan G. Sarafianos[1,3,6]

[1]Bond Life Sciences Center, [2]Chemistry, [3]Molecular Microbiology & Immunology, University of Missouri, Columbia, MO; [4]Physics & Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL; [5]Pathology, Microbiology & Immunology, Vanderbilt University, School of Medicine, Nashville, TN; [6]Biochemistry, University of Missouri, Columbia, MO; [7]Structural Biology, University of Pittsburgh, School of Medicine, Pittsburgh, PA

During HIV-1 maturation, CA proteins assemble into a conical capsid surrounding the viral genome. The structure of CA and its effects on capsid stability are critical for uncoating, reverse transcription, nuclear entry, integration site selection, and assembly. CA folds into two distinct domains, N-terminus (CA_{NTD}) and C-terminus (CA_{CTD}), which are connected by a linker. A number of mutations in CA are shown to alter the intrinsic stability of the HIV-1 capsid and are associated with impaired infectivity. Among them, the P38A CA mutants exhibit reduced stability and are impaired for reverse transcription. In contrast, the E45A mutation results in hyperstable capsids that undergo normal reverse transcription but delayed uncoating. Notably, compensatory CA mutations T216I and R132T partially rescue virus infection from the defects associated with the P38A and E45A mutations, respectively. While NMR analysis of CA_{NTD} protein fragments showed that the P38A, E45A and R132T CA mutations cause localized chemical shift changes, the exact structural effect of the mutations in the context of full CA and capsid assemblies remained unclear.

To address this, we have solved the crystal structures of full-length CA with P38A, P38A/T216I, E45A or E45A/R132T substitutions in wild type (WT) CA. Crystallization in space group P6 (one molecule per asymmetric unit) enables examination of mutation effects on CA-CA interactions. Compared to WT CA, the structures of all four CA mutants vary at the regions of the changed residues and in some cases we observe changes that are remote to the mutation sites. In the P38A CA structure, changes are primarily localized at the mutation site in helix 2, but they appear to also be transmitted to neighboring helix 1 and the loop between these two helices. Those rearrangements are similarly observed in P38A/T216I, together with other subtle changes that appear to be associated with the T216I mutation. Thus, P38A and P38A/T216I affect CA_{NTD}-CA_{NTD} and CA_{NTD}-CA_{CTD} interactions in the hexamer.

Surprisingly, E45A crystallizes in the same space group, but with two different unit cell dimensions: a=b=87.6 Å, c=56.6 Å and a=b=92.5 Å, c=57.8 Å. The former induces N-terminal interactions at the 3-fold inter-hexamer symmetry axes that involve R82 of the CA_{NTD}. Altered interactions are also observed at the 2-fold and 3-fold CA_{CTD} interfaces that are distant to the location of E45A. These changes are essentially reversed in the E45A/R132T CA structure. The latter E45A structure exhibits mostly localized changes at the site of mutation. We observe the electron density and can model beta-hairpin in the two E45A structures. Interestingly, it assumes two different conformations: in the former it goes in the clockwise direction ('open'), while in the latter – counterclockwise ('closed'). Collectively, the structures support the hypothesis that CA plasticity is a key factor for its stability and illustrate the challenges of understanding the effect of even single mutations on this highly flexible protein.

F63. Functionally Important Dynamics in HIV-1 Capsid Assemblies: Atomic-Level Understanding by Integrated MAS NMR, MD, and Density Functional Theory

Huilan Zhang[1,2], Rupal Gupta[1,2], Guangjin Hou[1,2], Manman Lu[1,2], Jinwoo Ahn[2,3], In-Ja Byeon[2,3], Christopher J. Langmead[4], Ivan Hung[5], Peter L. Go'kov[5], Zhehong Gan[5], William Brey[5], Marc Caporini[6], Melanie Rosay[6], Werner Maas[6], Jochem Struppe[6], David A. Case[7], Hartmut Oschkinat[8], Guido Pintacuda[9], Anne Lesage[9], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,2]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 5th Ave., Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA; [4]Computer Science Department, Carnegie Mellon University, 5000 Forbes Avenue, Pittsburgh, PA; [5]National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL; [6]Bruker Biospin Corporation, 15 Fortune Drive, Billerica, MA; [7]BioMaPS Institute and Department of Chemistry and Chemical Biology, Rutgers University, 147 Frelinghuysen Road, Piscataway, NJ; [8]Leibniz-Institut für Molekulare Pharmakologie, Robert-Roessle-Str. 10, 13125 Berlin, Germany; [9]Centre de RMN à Très Hauts Champs, UMR 5280 CNRS / Ecole Normale Supérieure de Lyon, 5 rue de la Doua, 69100 Villeurbanne (Lyon), France

The lifecycle and infectivity of human immunodeficiency virus (HIV-1) are modulated by the structure[1] and dynamics[2] of the mature capsid assemblies. Recent MAS NMR studies from our laboratory have demonstrated that interaction of CA assemblies with the host factor Cyclophilin A (CypA) is regulated by the dynamic allostery of the CypA binding loop in CA, suggesting that these motions at various timescales play an important role in HIV assembly, interactions with host factors and infectivity.[2] We present an integrated approach to gain atomic-level access to the dynamics in HIV-1 assemblies using MAS NMR, molecular dynamics (MD) simulations and density functional theory (DFT) calculations. We have probed the internal backbone dynamics in CA tubular assemblies using isotropic and anisotropic components of ^{13}C and ^{15}N chemical shifts. The ^{13}C and ^{15}N chemical shifts anisotropy (CSA) tensors are found to be sensitive reporters of the motions occurring on timescales of nano- to microseconds. At room temperature, due to molecular motions, the observed CSA tensors exhibit a reduced magnitude and thereby act as site-specific markers of dynamics. The dynamically averaged CSA tensors computed by DFT from substructures of MD trajectories are found to be in remarkable agreement with the experimental NMR results, and reveal that the relative orientations of the tensors undergo significant changes, while the principal components experience only very small variations. We also demonstrate that by using advanced sensitivity-enhanced MAS NMR techniques at cryogenic temperatures, namely dynamic nuclear polarization (DNP), the conformational space adopted by individual CA residues can be probed. The isotropic chemical shift distributions manifested in the lineshapes in 2D heteronuclear DNP-enhanced MAS NMR spectra provide insight into the conformational heterogeneity of dynamic domains, such as the CypA-binding loop residues, and can be predicted by MD simulations. Taken together, we show that an integrated analysis combining MAS NMR experiments and computational methodologies can provide in-depth, atomic-level information on the internal motions and their contributions to local structure, which may play a vital role in the proliferation and infectivity of HIV-1 virions. This work was supported by the National Institute of Health (P50GM082251) and is a contribution from the Pittsburgh Center for HIV Protein Interaction. We acknowledge the support of the NSF CHE0959496 grant for acquisition of the 850 MHz NMR spectrometer and of the NIH P30GM110758-01, P30GM103519 grants for the support of core instrumentation infrastructure at the University of Delaware.

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F64. Toward Atomic-Resolution Structure of Conical CA A204C Assemblies by Magic Angle Spinning NMR

Xingyu Lu[1,2], Guangjin Hou[1,2], Huilan Zhang[1,2], Manman Lu[1,2], Mingzhang Wang[1,2], Jinwoo Ahn[2,3], In-Ja L. Byeon[2,3], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,2]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 5th Ave., Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA

The mature HIV capsid is a conical shell that is formed during viral maturation by the assembly of ~1500 capsid protein (CA) molecules, which are organized into ~256 hexamers and 12 pentamers. It is widely accepted that the presence of pentamers induces the curvature necessary to form the cone shape of the capsid.[1] In vitro, the HIV-1 CA A204C mutant assembles into stable conical structures of homogeneous size, unlike the wild type CA that yields tubular assemblies.[2] To understand the atomic-resolution structure of the conical assemblies of CA A204C mutant, we have employed multidimensional magic angle spinning (MAS) NMR spectroscopy, which is well suited for atomic-level investigations into structure and dynamics of HIV-1 CA capsid protein assemblies, as we have shown previously.[3] We have collected an exhaustive series of two- and three-dimensional MAS NMR spectra of CA A204C conical assemblies, from which we have assigned ca. 80% of the carbon and nitrogen chemical shifts. We have analyzed the conformational changes induced upon the A204C mutation, by examining the chemical shift perturbations with respect to the tubular assemblies of wild type CA. We have examined the residue-specific conformational dynamics occurring on the nanosecond to millisecond timescales in A204C conical assemblies, by recording the ^{1}H-^{15}N and/or ^{1}H-^{13}C dipolar order parameters. The analysis reveals changes in the residue-specific motions in conical assemblies of A204C CA vs. the tubular assemblies of wild type CA. This work was supported by the National Institute of Health (P50GM082251) and is a contribution from the Pittsburgh Center for HIV Protein Interaction. We acknowledge the support of the NSF CHE0959496 grant for acquisition of the 850 MHz NMR spectrometer and of the NIH P30GM110758-01, P30GM103519 grants for the support of core instrumentation infrastructure at the University of Delaware.

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F65. Chemical Nature and Physical Properties of the HIV-1 Capsid from All-Atom Molecular Dynamics Simulations

Juan R Perilla and Klaus Schulten

University of Illinois at Urbana-Champaign, Physics and Beckman Institute, Urbana, IL

The HIV capsid is large, containing about 1,300 proteins with altogether 4 million atoms. Although the capsid proteins are all identical, they nevertheless arrange themselves into a largely asymmetric structure. The large size and lack of symmetry pose a huge challenge to studying the chemical details of the HIV capsid. Simulations of 64 million atoms for over 1 micro-second allow us to conduct a comprehensive study of the physical properties of the entire HIV capsid including its electrostatic potential, all-atom normal modes, as well as the effects of the solvent, namely ions and water, on the capsid. In addition, we are able to characterize different regions of the capsid according to its acoustic properties. The results from the simulations reveal critical details regarding the dynamics of the capsid with important implications for assembly, uncoating and nuclear import.

F66. Virtual Screening of HIV-1 Mature Capsid Protein

Pierrick Craveur, Stefano Forli, and Arthur Olson

Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, California, United States of America

The mature capsid proteins (CA) complex in homo-hexamers and homo-pentamers, which assemble to form the capsid core (Briggs and Krausslich 2011). Mutagenesis experiments revealed that core stability is fine-tuned to allow ordered disassembly during early stage of virus replication cycle (Forshey et al. 2002). This leads to recent efforts on drug development, targeting the mature capsid protein, and acting either as stabilizer or destabilizer of the core (Thenin-Houssier and Valente 2016). To date, no CA-inhibitors are approved for clinical use. With the goal of identifying novel active compounds, we set up a large virtual screening (VS) campaign in collaboration with the Sarafianos's lab (University of Missouri, Columbia).

Based on the crystal structure of the native hexameric capsid protein (PDB: 4xfx), recently solved by Sarafianos's lab, we performed structural analysis on a pocket involving at the interface of two monomers in the hexameric complex. In order to assess structural variability in this region, six additional CA X-ray structures from the PDB (including apo and holo forms), and the full capsid core model (1,296 chains) assembled by Schulten's lab, were used. The structural analysis at this interface highlights the high degree of rigidity of the backbone and the large range of structural changes of the side chains. The pocket of interest overlaps the biding site of 3 known ligands (BI-1, BI-2, PF74). The determined X-ray poses of these ligands has been used as a control to identify which structures resulted in highest success rates in re-docking experiments. Based on this analysis, we defined a set of 8 structures with specific combinations of flexible residues, and used them as targets for the virtual screening computation.

A library of ~60K compounds from subsets of ZINC database (Sterling and Irwin 2015) were used, containing commercially available substances and FDA approved drugs. To minimize the false positive rate, due of both search and scoring issues, 5 replicas of each docking experiments were computed. The complete VS represents a total of ~1.8 M docking computations with the VINA software (Trott and Olson 2010).

Sixty compounds presenting consistencies across the replicas and the targets were selected for experimental assays.

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F67. Dynamic Allostery Governs Cyclophilin A - HIV-1 Capsid Interplay

Manman Lu[1,2], Guangjin Hou[1,2], Huilan Zhang[1,2], Christopher L. Suiter[1,2], Jinwoo Ahn[2,3], In-Ja L. Byeon[2,3], Juan R. Perilla[4], Christopher J. Langmead[5], Ivan Hung[6], Peter L. Gor'kov[6], Zhehong Gan[6], William Brey[6], Christopher Aiken[2,7], Peijun Zhang[2,3], Klaus Schulten[4], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,2]

[1]Department of Chemistry and Biochemistry, University of Delaware, Newark, DE; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 5th Ave., Pittsburgh, PA; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA; [4]Center for Biophysics and Computational Biology and Beckman Institute for Advanced Science and Technology and Departments of Physics, Chemistry, and Biochemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois; [5]School of Computer Science, Computational Biology Department, Carnegie Mellon University, 5000 Forbes Ave., Pittsburgh, PA; [6]National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL; [7]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN

In a mature HIV-1 virion, the viral CA protein assembles into a conical capsid, enclosing the viral genome[1]. The host cell protein cyclophilin A (CypA) binds the capsid directly and regulates viral infectivity by an unknown mechanism[2]. Two CA mutants A92E and G94D tolerate CypA inhibition and are known as CypA escape mutants[3]. We have addressed the role of conformational dynamics on the nanosecond to millisecond timescales in the escape from CypA dependence by magic angle spinning (MAS) NMR and molecular dynamics (MD)[4]. ^{1}H-^{15}N and ^{1}H-^{13}C dipolar order parameters (S) obtained from MAS NMR experiments on CA assemblies, CypA escape mutants A92E and G94D, and CA/CypA assemblies are in quantitative agreement with those calculated from MD trajectories[4]. Our data demonstrate that CA assemblies are dynamic on multiple timescales, especially in the CypA binding loop[4]. These motions are significantly reduced in CA/CypA assemblies[4]. Remarkably, the CypA escape mutant assemblies exhibit dynamic behavior similar to that in the CA/CypA assemblies[4]. Together, these findings suggest that dynamic allostery mechanism may govern the CA escape from CypA dependence[4]. To study the interfaces of interaction between CA and CypA, we examined a series of CA/CypA assemblies, where either CA or CypA were uniformly ^{13}C, ^{15}N labeled[5]. Multiple chemical shift perturbations and intensity changes were observed upon formation of CA/CypA assemblies at different CA:CypA ratios. Interestingly, while many spectral changes map onto CA and CypA residues comprising the canonical binding sites, a large number of perturbations are associated with residues distal to these canonical binding sites, indicating either additional binding modes, allosteric effects, or both[5]. CryoEM and MD studies reveal that CypA binds to CA by selectively bridging the CA dimer along the direction of highest curvature[5]. This work was supported by the National Institute of Health (P50GM082251) and is a contribution from the Pittsburgh Center for HIV Protein Interaction. We acknowledge the support of the NSF CHE0959496 grant for acquisition of the 850 MHz NMR spectrometer and of the NIH P30GM110758-01, P30GM103519 grants for the support of core instrumentation infrastructure at the University of Delaware.

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F68. Expression and Purification of Pentameric Vpu

Yi-Liang Liu[1], Ignacio Asial[1,2], and Robert M. Stroud[1]

[1]Department of Biochemistry and Biophysics, University of California, San Francisco, California, USA; [2]School of Biological Sciences, Nanyang Technological University, Singapore, Singapore

Viral Protein U (Vpu) plays a vital role in associating with host proteins to initiate downstream interactions. Vpu is the only transmembrane protein among the four accessory proteins existing in HIV protein assembly. It is responsible for downregulating several host factors including CD-4, the receptor for entry, and restriction factor BST-2 (tetherin) which facilitates entry and release of viral particles, respectively. It does so through the E3 ligase system (Cul1-βTrCP-Skp1-Rbx1). Most importantly, it acts functionally as a viroporin in a homopentamer to control the membrane potential and regulate membrane permeability. This behavior results in an intermediate step to facilitate the entry and release of virions.

We have succeeded in expressing Vpu using a new approach, a state-of-the-art method to randomly select Vpu mutants, that guided us to dramatically improve the expression level in bacteria by at least 1,000 fold (judging from the anti-pentaHis Ab blot data). This mutant can be solubilized by various detergents including DDM and enables us to perform more extensive screens on its stability and states of oligomerization. We also carried out detergent and pH screens to further stabilize the protein and achieve high quality of pentameric Vpu. The formation of different oligomerization states can be well handled by varying detergents and pHs. We are able to express pure pentameric Vpu in a quantity of 0.5mg/L bacterial culture. Negative stain EM images also suggest a pentameric-like species.

F69. Arrayed Analysis of Immune Evasion: High Content Imaging Screen Reveals Novel Targets of HIV-1 Vpu

Guney Boso, Prashant Jain, Paul de Jesus, Quy Nguyen and Sumit Chanda

Sanford Burnham Prebys Medical Discovery Institute

One of the mechanisms by which HIV-1 evades immune restriction is through targeting of interferon stimulated genes (ISGs) such as APOBEC3G and BST-2 via viral accessory proteins. In an effort to test the hypothesis that there are additional ISGs that are targeted by HIV-1, we developed a high content imaging platform that enables the arrayed analysis of the stability of proteins in the presence or absence of a second protein. We assessed the performance of this platform to identify novel cellular targets of HIV-1 Vpu. We co-transfected 950 V5-tagged ISGs into HEK293 cells together with FLAG-tagged Vpu, or LacZ. Cells were then analyzed for V5 and FLAG staining intensities to reveal 40 proteins that were significantly destabilized after the expression of Vpu, but not LacZ. We confirmed degradation of a subset of these hits in the same system by immunoblotting analysis +/- proteasome inhibitor. We also found that at least five of these proteins are degraded in the HIV-1 infected cells in a Vpu dependent manner. One of these hits, UBE2L6 is an E2 ligase for ISG15 conjugation. Interestingly, we observed a Vpu dependent decrease in the levels of ISG15 conjugates in multiple cell types suggesting that HIV-1 targets this antiviral mechanism as an immune evasion strategy. Moreover we showed that overexpression of two of the hits (CD99 and PLP2) lead to a substantial decrease in HIV-1 infectivity. Our studies suggest that CD99 and PLP2 restrict viral infectivity by causing production of viral particles that are defective in cellular binding/entry.

F70. Studies of Membrane-Bound HIV Nef to Elucidate its Role in T-Cell Activation

Mathias Lösche[1,2], Michael S. Kent[3], and Thomas E. Smithgall[4]

[1]Dept. of Physics and Dept. of Biomedical Engineering, Carnegie Mellon University; [2]The NIST Center for Neutron Research; [3]Sandia National Laboratories; and [4]Dept. of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine

Despite its designation as an 'accessory' protein encoded by primate lentiviruses such as HIV and SIV, Nef is a determining factor in immune evasion of infected cells as well as AIDS progression.[1] Nef drives HIV-1 pathogenesis by interacting with a multitude of host cell factors, resulting in downregulation of viral (CD4, CXCR4, CCR5) and immune (MHC-I/II) receptors[2] as well as constitutive activation of non-receptor protein kinases of the Src and Tec families.[3] These Nef functions are completely dependent upon membrane localization, which involves an N-terminal myristoyl group as well as a conserved basic patch near the N-terminus. Despite the membrane dependence of these interactions for Nef function, the influence of the lipid bilayer on the three-dimensional structures of these critical host-pathogen protein complexes remain elusive. Recent studies have characterized myristoylated Nef on membrane-mimetic Langmuir monolayers by neutron and x-ray reflection.[4,5] These studies showed that myristoylation is required for Nef membrane insertion and that the protein adopts distinct conformations dependent on the extent of insertion.[4,6] We propose to extend these studies by characterizing the structural and dynamic properties of Nef on sparsely-tethered bilayer lipid membranes (stBLMs)[7] on solid substrates, a fluid membrane model system with physical properties similar to free lipid membranes.[8] Preliminary data indicate that we can immobilize the membrane-binding SH4–U (Src homology 4 and Unique) segments of Hck, a Src-family kinase expressed in HIV target cells that interacts strongly with Nef.[3] Similarly, we are characterizing the PH domain-mediated membrane binding of Itk, a Tec-family kinase critical for T-cell activation[9] that has also been linked to the HIV-1 life cycle through Nef.[10,11] Our ultimate goal is to understand the structural relationship between Nef and partner kinases in the membrane environment, the impact of the membrane on Nef dimerization, which is also critical to function,[12] and Nef-induced kinase activation. Our initial progress toward these aims will be reported.

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List of Participants

Sherimay Ablan
CCR/NCI
Email: sablan@mail.nih.gov

Cameron Abrams
Drexel University
Email: CFA22@DREXEL.EDU

Kriti Acharya
Drexel University College of Medicine
Email: acharyakriti5@gmail.com

Nowah Afangbedji
Howard University
Email: Nowah12@gmail.com

Adel Ahmed
Drexel University College of Medicine
Email: adel.ahmed@drexelmed.edu

Jinwoo Ahn
University of Pittsburgh
Email: jia12@pitt.edu

Koree Ahn
Thomas Jefferson University
Email: koree.ahn@jefferson.edu

Chris Aiken
Vanderbilt University Medical Center
Email: chris.aiken@vanderbilt.edu

Akbar Ali
University of Massachusetts Medical School
Email: akbar.ali@umassmed.edu

Akram Alian
Technion - Israel Institute of Technology
Email: alian@tx.technion.ac.il

Atef Allam
Walter Reed (MHRP)
Email: AAllam@hivresearch.org

Zandrea Ambrose
University of Pittsburgh
Email: zaa4@pitt.edu

Mark Andrake
Fox Chase Cancer Center
Email: mark.andrake@fccc.edu

Isaac Angert
University of Minnesota
Email: angert@physics.umn.edu

Eddy Arnold
CABM and Rutgers University
Email: arnold@cabm.rutgers.edu

soheila Bahmanjah
NIH
Email: soheila.bahmanjah@nih.gov

Hongjun Bai
US Military HIV Research Program, HJF
Email: hbai@hivresearch.org

Janae Baptiste
University of Maryland, Baltimore County
Email: jbaptiste@umbc.edu

Joseph Bauman
Rutgers University
Email: bauman@cabm.rutgers.edu

Owen Becette
University of Maryland College Park
Email: obecette@umd.edu

Gregory Bedwell
University of Alabama at Birmingham
Email: gbedwell@uab.edu

Richard Belew
Univ. California -- San Diego
Email: rbelew@ucsd.edu

Paul Bieniasz
Rockefeller University
Email: pbienias@adarc.org

Jennifer Binning
University of California, San Francisco
Email: jen.binning@gmail.com

Pamela Bjorkman
California Institute of Technology
Email: bjorkman@caltech.edu

Marina Bleck
The Rockefeller University
Email: fixm@rockefeller.edu

Hal Bogerd
Duke University
Email: hbogerd@hotmail.com

Jennifer Bohn
University of Michigan
Email: jenbohn@umich.edu

Daniel Bolon
University of Massachusetts Medical School
Email: Dan.Bolon@umassmed.edu

Mario Borgnia
National Cancer Institute
Email: mborgnia@nih.gov

Kathleen Boris-Lawrie
Univ Minnesota
Email: kbl@umn.edu

Guney Boso
Sanford Burnham Prebys Medical Discovery Institute
Email: gboso@sbpdiscovery.org

Jeffrey Boucher
University of Massachusetts Medical School
Email: jeffrey.boucher@umassmed.edu

Kate Bredbenner
Rockefeller University
Email: kate.bredbenner@gmail.com

Melanie Brewer
UCSF
Email: melanie.brewer@ucsf.edu

Teresa Brosenitsch
University of Pittsburgh School of Medicine
Email: tab24@pitt.edu

William Brown
University of Michigan
Email: wclayb@umich.edu

ANISSA BROWN
NIH
Email: BROWNANI@MAIL.NIH.GOV

Joshua Brown
University of Maryland Baltimore County
Email: jdbrown@umbc.edu

Cleo Burnett
University of Michigan
Email: cleob@umich.edu

Frederic Bushman
University of Pennsylvania
Email: bushman@mail.med.upenn.edu

Chang Byeon
University of Pittsburgh
Email: Chb49@pitt.edu

Guillermo Calero
University of Pittsburgh
Email: guc9@pitt.edu

William Cantara
The Ohio State University
Email: wcantara@gmail.com

Lars-Anders Carlson
UC Berkeley
Email: lacarlson@berkeley.edu

Stacy Carrington-Lawrence
NIH Office of the Director
Email: carringtons@od.nih.gov

Stephen Carter
Caltech
Email: scarter@caltech.edu

David Case
Rutgers University
Email: david.case@rutgers.edu

Irwin Chaiken
Drexel University College of Medicine
Email: ichaiken@drexelmed.edu

Sumit Chanda
Sanford Burnham Prebys Medical Research Institute
Email: schanda@sbpdiscovery.org

Sumit Chanda
Sanford Burnham Prebys
Email: schanda@burnham.org

Bin Chen
University of Maryland
Email: bchen1@umd.edu

Eunice Chen
Penn State College of Medicine
Email: echen@hmc.psu.edu

Jianbo Chen
NCI
Email: chenjia@mail.nih.gov

Jae Eun Cheong
Temple University
Email: Jcheong@temple.edu

Chelsy Chesterman
Rutgers University
Email: chesterman@cabm.rutgers.edu

Jean Chin
NIH-NIGMS
Email: chinj@nigms.nih.gov

Jakub Chrustowicz
University of Virginia
Email: jakub.chrustowicz@gmail.com

Hui-Yi Chu
Scripps
Email: huiyichu@scripps.edu

Hema Chug
Harvard Medical School
Email: hchug@crystal.harvard.edu

Elizabeth Church
NIAID
Email: elizabeth.church@nih.gov

John Coffin
Tufts University
Email: john.coffin@tufts.edu

Roopa Comandur
The Ohio State University
Email: comandur.1@buckeyemail.osu.edu

Mauricio Comas-Garcia
NCI
Email: mauricio.comasgarcia@nih.gov

Michael Conlon
NIAID
Email: michael.conlon@nih.gov

Charles Craik
UCSF
Email: charles.craik@ucsf.edu

Pierrick Craveur
The Scripps Research Institute
Email: pcraveur@scripps.edu

Daniel Cyburt
University of Missouri
Email: dwcxc5@mail.missouri.edu

Kalyan Das
Rutgers University
Email: kalyan@cabm.rutgers.edu

Debananda Das
NCI/NIH
Email: dasd@mail.nih.gov

Chandravanu Dash
Meharry Medical College
Email: cdash@mmc.edu

Siddhartha Datta
NCI
Email: dattasi@mail.nih.gov

T. Kwaku Dayie
University of Maryland
Email: dayie@umd.edu

Asim Debnath
New York Blood Center
Email: adebnath@nybc.org

Maria DeLucia
University of Pittsburgh
Email: mld80@pitt.edu

Nanjie Deng
Temple University
Email: nanjie.deng@gmail.com

YiSong Deng
The Scripps Research Institute
Email: katrinas@scripps.edu

BARNA DEY
CSR/NIH
Email: bdey@mail.nih.gov

Robert Dick
Cornell University
Email: rad82@cornell.edu

Ira Dicker
ViiV Healthcare
Email: ira.b.dicker@viivhealthcare.com

Pengfei Ding
University of Maryland Baltimore County
Email: dingp@umbc.edu

Victoria DSouza
Harvard University
Email: dsouza@mcb.harvard.edu

Vincent Dussupt
MHRP
Email: vdussupt@hivresearch.org

Charles Edmonds
NIH/NIGMS
Email: edmondsc@nigms.nih.gov

Alan Embry
NIAID
Email: embrya@mail.nih.gov

Ann Emery
UNC Chapel Hill
Email: aemery@email.unc.edu

Alan Engelman
Dana-Farber Cancer Institute
Email: alan_engelman@dfci.harvard.edu

Nicholas Farrell
Virginia Commonwealth University
Email: npfarrell@vcu.edu

Jerry Feigenson
Cornell University
Email: gwf3@cornell.edu

Daniela Fera
Boston Children's Hospital /Harvard Medical School
Email: fera@crystal.harvard.edu

Melissa Fernandez
NIH-NCI
Email: melissa.fernandez2@nih.gov

Diana Finzi
NIAID
Email: dfinzi@nih.gov

Douglas Fischer
University of Pittsburgh School of Medicine
Email: dkf12@pitt.edu

William Flynn
Rutgers University
Email: wflynny@gmail.com

Brian Fochtman
Stony Brook University
Email: brianfochtman@gmail.com

Stefano Forli
The Scripps Research Institute
Email: forli@scripps.edu

Alan Frankel
UCSF
Email: frankel@cgl.ucsf.edu

Eric Freed
NCI
Email: efreed@nih.gov

Ernesto Freire
Johns Hopkins University
Email: ef@jhu.edu

Robert Freund
Center for Scientifc Review, NIH
Email: freundr@csr.nih.gov

Fred Friedman
NIH
Email: fred.friedman@nih.gov

Sarah Fritz
Antioch College and The Ohio State University
Email: sfritz@antiochcollege.org

Althea Gaffney
University of Pennsylvania
Email: agaffney@sas.upenn.edu

Christy Gaines
HHMI at UMBC
Email: cgaines1@umbc.edu

Laura Ganser
Duke University
Email: laura.ganser@duke.edu

Barbie Ganser-Pornillos
University of Virginia
Email: bpornillos@virginia.edu

Larry Gerace
The Scripps Research Institute
Email: lgerace@scripps.edu

Stephen Goff
Columbia University
Email: spg1@cumc.columbia.edu

David Goodsell
The Scripps Research Institute
Email: goodsell@scripps.edu

Darnell Graham
UNC Chapel Hill
Email: wdgram79@email.unc.edu

Melissa Grenier
University of Pennsylvania
Email: grenier@sas.upenn.edu

Anna Gres
University of Missouri-Columbia
Email: ag32d@mail.missouri.edu

John Grime
The University of Chicago
Email: jgrime@uchicago.edu

Angela Gronenborn
University of Pittsburgh
Email: amg100@pitt.edu

John Gross
University of California, San Francisco (UCSF)
Email: jdgross@cgl.ucsf.edu

Jiaye Guo
Stony Brook University
Email: jiaye.guo@stonybrook.edu

Kushol Gupta
Perelman School of Medicine - Univ. of PA
Email: kgupta@upenn.edu

Rupal Gupta
University of Delaware
Email: rupalg@udel.edu

Sebanti Gupta
NIH
Email: sebantigupta@gmail.com

John Hammond
The Scripps Research Institute
Email: hammondj@scripps.edu

John Hammond
The Scripps Research Institute
Email: katrinas@scripps.edu

Amanda Hargrove
Duke University
Email: amanda.hargrove@duke.edu

Reuben Harris
Howard Hughes Medical Institute
Email: rsh@umn.edu

Audray Harris
NIH
Email: harrisau@mail.nih.gov

Jerry Joe Harrison
Rutgers, The State University of New Jersey
Email: jerryjoe.harrison@rutgers.edu

Frank Heinrich
Carnegie Mellon University
Email: fheinrich@cmu.edu

Xiao Heng
University of Missouri, Columbia
Email: hengx@missouri.edu

Alon Herschhorn
Dana-Farber Cancer Institute
Email: alon_herschhorn@dfci.harvard.edu

Andrew Holmes
Drexel University College of Medicine
Email: aph52@drexel.edu

Iris Hood
NIH
Email: iris.v.hood@gmail.com

Thomas Hope
Northwestern University
Email: thope@northwestern.edu

Steven Horner
University of Rochester School of Medicine & Denti
Email: Steven_Horner@urmc.rochester.edu

Shurong Hou
UMass medical school
Email: Shurong.Hou@umassmed.edu

Wei-Shau Hu
National Cancer Institute
Email: Wei-Shau.Hu@nih.gov

Ivy Huang
Yale University
Email: pei-tzu.huang@yale.edu

Stephen Hughes
NCI at Frederick
Email: hughesst@mail.nih.gov

James Hurley
UC Berkeley
Email: jimhurley@berkeley.edu

Rieko Ishima
University of Pittsburgh School of Medicine
Email: ishima@pitt.edu

Michelle Itano
The Rockefeller University
Email: michelle.itano@gmail.com

Janet Iwasa
University of Utah
Email: jiwasa@biochem.utah.edu

Niyati Jain
CWRU
Email: jxn172@case.edu

Sanath Kumar Janaka
NCI
Email: sanath.janaka@nih.gov

Grant Jensen
Caltech
Email: jensen@caltech.edu

Daniel Johnson
Rockefeller University
Email: djohnson@rockefeller.edu

Bruce Johnson
CUNY Advanced Science Research Center
Email: bruce.johnson@asrc.cuny.edu

Jeffrey Johnson
UCSF
Email: jeffrey.johnson@ucsf.edu

Christopher Jones
NIH
Email: jones.cprice@gmail.com

Robyn Kaake
The J. David Gladstone Institutes
Email: robyn.kaake@gladstone.ucsf.edu

Virginia Kan
VA Medical Center
Email: virginia.kan@va.gov

Tapan Kanai
National Cancer institute(NCI)
Email: tapan.kanai@nih.gov

Jonathan Karn
Case Western Reserve University
Email: jonathan.karn@case.edu

Michael Kay
University of Utah
Email: kay@biochem.utah.edu

Sarah Keane
HHMI/UMBC
Email: keanes@umbc.edu

Vineet KewalRamani
NCI
Email: vineet@mail.nih.gov

Shahid Khan
University of Pittsburgh
Email: shk88@pitt.edu

Zaigham Khan
Fox Chase Cancer Center / Temple University
Email: zaigham.khan@fccc.edu

Siarhei Kharytonchyk
University of Michigan
Email: siarheik@umich.edu

Mukta Khasnis
Thomas Jefferson University
Email: mdk013@jefferson.edu

Baek Kim
Emory University
Email: baek.kim@emory.edu

Karen Kirby
University of Missouri
Email: kirbyk@missouri.edu

Sharon Kirk
University of Pennsylvania
Email: sharonma@sas.upenn.edu

Paul Kitsutani
NIH/NIAID/DAIDS
Email: paul.kitsutani@nih.gov

Matthew Kobe
Ohio State University
Email: kobe.16@osu.edu

Douglas Kojetin
The Scripps Research Institute, Scripps Florida
Email: dkojetin@scripps.edu

Takahide Kono
U Mass Med School
Email: Takahide.Kono@umassmed.edu

Hamed Kooshapur
NIH
Email: hamed.kooshapur@nih.gov

Dima Kozakov
Stony Brook
Email: midas@laufercenter.org

Artem Krantsevich
Stony Brook University
Email: akrantsevich@gmail.com

Nevan Krogan
University of California
Email: nevan.krogan@ucsf.edu

Troy Krzysiak
University of Pittsburgh
Email: trk8@pitt.edu

Tonya Kueck
The Rockefeller University
Email: tkueck@adarc.org

Namita Kumari
Howard University
Email: namita.kumari@howard.edu

Lillian Kuo
NIAID
Email: Lillian.Kuo@nih.gov

Nese Kurt Yilmaz
UMass Medical School
Email: Nese.KurtYilmaz@umassmed.edu

Mamuka Kvaratskhelia
The Ohio State University
Email: kvaratskhelia.1@osu.edu

Yen-Ting Lai
NIH
Email: yen-ting.lai@nih.gov

Rajan Lamichhane
The Scripps Research Institute
Email: rajan@scripps.edu

Hatajai Lassiter
Howard University
Email: hatajai.lassiter@bison.howard.edu

Stuart Le Grice
National Cancer Institute
Email: legrices@mail.nih.gov

Kelly Lee
University of Washington
Email: kklee@uw.edu

Sook-Kyung Lee
University of North Carolina
Email: sooklee@med.unc.edu

Florian Leidner
University of Massachusetts Medical Schoo
Email: florian.leidner@umassmed.edu

Jeffrey Levengood
Case Western Reserve University
Email: jdl108@case.edu

Judith Levin
NIH
Email: levinju@mail.nih.gov

Ronald Levy
Temple University
Email: ronlevy@temple.edu

Catherine Lewis
NIH
Email: lewisc@nigms.nih.gov

Shuang Li
NIH/NIDDK
Email: shuang.li@nih.gov

Xindi Li
Stony Brook University
Email: xindi.li@stonybrook.edu

Christopher Lim
Yale University
Email: christopher.lim@yale.edu

Jared Lindenberger
The Ohio State University
Email: lindenberger.10@osu.edu

Qingbo Liu
NIAID
Email: qingbo.liu@nih.gov

Bindong Liu
Meharry Medical College
Email: bliu@mmc.edu

Yi-Liang Liu
University of California, San Francisco
Email: lucas.liu@msg.ucsf.edu

Xi Liu
UCSF
Email: liuxitianping0921@gmail.com

Yang Liu
NCI DRP Program
Email: liuy18@nih.gov

Gordon Lockbaum
University of Massachusetts Medical School
Email: gordon.lockbaum@umassmed.edu

Andrew Longhini
University of Maryland, College Park
Email: longhini@umd.edu

Manman Lu
University of Delaware
Email: lumm@udel.edu

Xingyu Lu
University of Delaware
Email: luxingyu@udel.edu

Sabrina Lusvarghi
NIDDK
Email: sabrinal@mail.nih.gov

Mathias Lösche
Carnegie Mellon University
Email: quench@cmu.edu

Xiaochu Ma
Yale University
Email: Xiaochu.ma@yale.edu

Tatsuya Maehigashi
Emory University
Email: t.maehigashi@emory.edu

Shrawan Kumar Mageswaran
California Institute of Technology
Email: m.shrawankumar@gmail.com

Atanu Maiti
Leidos Biomedical Research, Inc/FNLCR
Email: atanu.maiti@nih.gov

Rebecca Maldonado
Penn State College of Medicine
Email: rjk297@psu.edu

João Mamede
Northwestern University
Email: joao.mamede@northwestern.edu

Lara Manganaro
Icahn School of Medicine at Mount Sinai
Email: lara.manganaro@mssm.edu

Louis Mansky
University of Minnesota
Email: mansky@umn.edu

Jan Marchant
UMBC
Email: janm@umbc.edu

Joseph Marcotrigiano
Rutgers University
Email: jmarco@cabm.rutgers.edu

Alex Marson
UCSF
Email: alexander.marson@ucsf.edu

Jessica Martin
University of Minnesota
Email: mart3243@umn.edu

Sergio Martinez
Rutgers University
Email: martinez@cabm.rutgers.edu

Takashi Masaoka
NCI-Frederick
Email: takashi.masaoka@nih.gov

Hiroshi Matsuo
Leidos Biomedical Research/FNLCR
Email: hiroshi.matsuo@nih.gov

David McDonald
Case Western Reserve University School of Medicine
Email: djm41@case.edu

Dwight McGee
Stony Brook University
Email: dwight.mcgee@gmail.com

Jennifer Meagher
University of Michigan
Email: jmeagher@umich.edu

Gretchen Meinke
Tufts University
Email: gretchen.meinke@tufts.edu

Morgan Meissner
University of Minnesota
Email: schuc041@umn.edu

Gregory Melikian
Emory University
Email: gmeliki@emory.edu

Peter Mercredi
St. Jude Children's Research Hospital
Email: peter.mercredi@stjude.org

David Millar
The Scripps Research Institute
Email: millar@scripps.edu

Roger Miller
NIAID, NIH
Email: rmiller@niaid.nih.gov

Jennifer Miller
FNLCR
Email: millerj@mail.nih.gov

Matthew Miller
Rutgers University
Email: mmiller@cabm.rutgers.edu

Francesca Moraca
Drexel University
Email: fm356@drexel.edu

Christopher Morgan
Case Western Reserve University
Email: cem137@case.edu

Walther Mothes
Yale University
Email: walther.mothes@yale.edu

Lubbertus Mulder
Ichan school of Medicine at Mount Sinai
Email: lubbertus.mulder@mssm.edu

Muhamad Mulwana
Orphans and Widows of AIDS Uganda
Email: sasintltd@yahoo.com

James Munro
Tufts University School of Medicine
Email: james.munro@tufts.edu

Karin Musier-Forsyth
Ohio State University
Email: musier@chemistry.ohio-state.edu

Mallory Myers
SoBran
Email: mallory.myers@nih.gov

Katherine Nakama
University of Michigan
Email: knakama@umich.edu

Hyeyeon Nam
University of Maryland
Email: hnam89@umd.edu

Aakansha Nangarlia
Drexel University
Email: an477@drexel.edu

Steevenson Nelson
NIH
Email: steevenson.nelson@nih.gov

Zhongzhen Nie
NIGMS
Email: niezhong@nigms.nih.gov

Olga Nikolaitchik
NCI
Email: nikolaio@mail.nih.gov

TJ Nitz
DFH Pharma
Email: tjnitz@comcast.net

Mariia Novikova
NCI Frederick
Email: maria.novikova@nih.gov

Julie Nyman
UMBC
Email: jnyman1@umbc.edu

Ina O'Carroll
US Naval Academy
Email: ocarroll@usna.edu

Dario Oliveira Passos
Salk Institute for Biological Studies
Email: dariorj@gmail.com

Erik Olson
Ohio State University
Email: erikolson9@gmail.com

Arthur Olson
The Scripps Research Institute
Email: olson@scripps.edu

Chiara Orlandi
University of Maryland
Email: corlandi@ihv.umaryland.edu

Nicole Orlovsky
Duke University
Email: nio@duke.edu

Lars Pache
Sanford Burnham Prebys Medical Discovery Institute
Email: lpache@sbpdiscovery.org

Savita Pahwa
University of Miami Miller School of Medicine
Email: spahwa@med.miami.edu

Chris Palmer
NIGMS
Email: crpalmer2009@gmail.com

Bibek Parajuli
Drexel University
Email: Bibek.Parajuli@DrexelMed.edu

Leslie Parent
Penn State College of Medicine
Email: lparent@psu.edu

Christian Parry
Center for Sickle Cell Disease
Email: christian.parry@Howard.edu

Vinay Pathak
National Cancer Institute
Email: vinay.pathak@nih.gov

Jean Patterson
Office of AIDS Research
Email: pattersj@od.nih.gov

Janet Paulsen
University of Massachusetts Medical School
Email: janet.paulsen@umassmed.edu

Srinivasa Penumutchu
Case Western Reserve University
Email: srp106@case.edu

Juan Perilla
University of Illinois at Urbana Champaign
Email: jrperillaj@gmail.com

Boris Peterlin
UCSF
Email: matija.peterlin@ucsf.edu

Erica Peterson
VCU
Email: ejpeterson@vcu.edu

Eva Petrakova
NIH/NIAID
Email: petrakoe@mail.nih.gov

Vincent Pham
Harvard University
Email: vincent_pham@alumni.brown.edu

Tatyana Polenova
University of Delaware
Email: tpolenov@udel.edu

Owen Pornillos
University of Virginia
Email: opornillos@virginia.edu

Lauren Prentis
Stony Brook Unviersity
Email: lep213@gmail.com

Peter Preusch
NIGMS
Email: preuschp@nigms.nih.gov

Peter Prevelige
Univ Alabama Birmingham
Email: prevelig@uab.edu

Roger Ptak
Southern Research Institute
Email: rptak@southernresearch.org

Botros Q
SUNY at Albany
Email: btoro@albany.edu

Caitlin Quinn
University of Delaware
Email: cmquinn@udel.edu

Sheikh Rahman
NCI
Email: sheikh.rahman@nih.gov

Jason Rausch
Frederick National Laboratory for Cancer Research
Email: rauschj@mail.nih.gov

Jonathan Rawson
National Cancer Institute
Email: jonathan.rawson@nih.gov

Krishanu Ray
University of Maryland School of Medicine
Email: kray@som.umaryland.edu

Thomas Reimonn
University of Massachusetts Medical School
Email: thomas.reimonn@umassmed.edu

Clifton Ricana
University of Missouri-Columbia
Email: clr6w7@mail.missouri.edu

Andrew Rice
Baylor College of Medicine
Email: arice@bcm.edu

Christopher Richards
University of Minnesota - Twin Cities
Email: rich1081@umn.edu

Brittany Rife
University of Florida
Email: brittany.rife@ufl.edu

Robert Rizzo
Stony Brook University
Email: rizzorc@gmail.com

Isaac Rodriguez-Chavez
NIDCR
Email: isaac@nidcr.nih.gov

Marcin Roganowicz
University of Virginia
Email: mdr3s@virginia.edu

Andrew Routh
University of Texas Medical Branch, Galveston
Email: alrouth@utmb.edu

Ioulia Rouzina
OSU
Email: irouzina@gmail.com

Francesc Ruiz
CABM - Rutgers University
Email: xavier@cabm.rutgers.edu

Linah Rusere
University of Massachusetts Medical School
Email: Linah.Rusere@umassmed.edu

Ryan Russell
University of Delaware
Email: russellr@udel.edu

Jamil Saad
UAB
Email: saad@uab.edu

Vincent Sabol
NIGMS
Email: sabolv@nigms.nih.gov

Saveez Saffarian
University of Utah
Email: saffarian@physics.utah.edu

Michael Sakalian
NIGMS
Email: sakalianm@mail.nih.gov

Stefan Sarafianos
University of Missouri
Email: sarafianoss@missouri.edu

Susan Schader
Southern Research
Email: sschader@southernresearch.org

Celia Schiffer
University of Massachusetts Medical School
Email: Celia.Schiffer@umassmed.edu

Arne Schon
Johns Hopkins University
Email: schon@jhu.edu

Ursula Schulze-Gahmen
UC Berkeley
Email: uschulze-gahmen@lbl.gov

Arnold Schwartz
George Washington University
Email: arnies@email.gwu.edu

Natalia Sevillano
UCSF
Email: natalia.sevillano@ucsf.edu

Nadine Shaban
University of Minnesota
Email: nmshaban@umn.edu

Stuart Shapiro
NIAID/DAIDS
Email: sshapiro@niaid.nih.gov

Woody Sherman
SchrÃ¶dinger
Email: woody.sherman@schrodinger.com

Chringma Sherpa
NCI
Email: chringma@gmail.com

Kishore Shetty
University of Illinois/ Heartland Health
Email: kshetty@heartlandalliance.org

Tania Silvas
UMass Medical School
Email: tania.silvas@umassmed.edu

Sanford Simon
Rockefeller University
Email: simon@rockefeller.edu

Viviana Simon
Icahn School of Medicine at Mount Sinai
Email: viviana.simon@mssm.edu

Anjali Singh
NIAID, NIH
Email: anjalisingh@niaid.nih.gov

Gatikrushna Singh
University Minnesota
Email: gsingh@umn.edu

Courtney Singleton
Stony Brook University
Email:
courtney.singleton@stonybrookmedicine.edu

Katarzyna Skorupka
University of Virginia
Email: ks2sd@virginia.edu

Jacek Skowronski
CWRU
Email: jacek.skowronski@case.edu

Ryan Slack
University of Pittsburgh, School of Medicine
Email: rls128@pitt.edu

Janet Smith
University of Michigan
Email: JanetSmith@umich.edu

Amber Smith
University of California, San Francisco
Email: amber.smith@ucsf.edu

Ward Smith
NIH/NIGMS
Email: ward.smith@nih.gov

Amos Smith, III
University of Pennsylvania
Email: smithab@sas.upenn.edu

Zhenwei Song
University of Missouri Columbia
Email: songzh@missouri.edu

Paul Spearman
Emory University
Email: paul.spearman@emory.edu

Ean Spielvogel
UNC Chapel Hill
Email: spielvoe@email.unc.edu

Robyn Stanfield
The Scripps Research Institute
Email: robyn@scripps.edu

Klaus Strebel
NIH, NIAID
Email: kstrebel@nih.gov

Madeleine Strickland
National Institutes of Health
Email: maddy.davison@nih.gov

Robert Stroud
University of California, San Francisco
Email: stroud@msg.ucsf.edu

Zhaoming Su
Baylor College of Medicine
Email: zhaoming.su@bcm.edu

Michael Summers
HHMI/UMBC
Email: summers@hhmi.umbc.edu

Brady Summers
Yale University
Email: brady.summers@yale.edu

Wes Sundquist
University of Utah
Email: wes@biochem.utah.edu

Ronald Swanstrom
UNC Chapel Hill
Email: risunc@med.unc.edu

Joanna Sztuba-Solinska
NIH/NCI
Email: joanna.sztuba-solinska@nih.gov

Matthew Takata
The Rockefeller University
Email: mtakata@rockefeller.edu

Alice Telesnitsky
University of Michigan
Email: ateles@umich.edu

Markus Thali
University of Vermont
Email: markus.thali@uvm.edu

Theresa Tiefenbrunn
TSRI
Email: theresat@scripps.edu

Blanton Tolbert
CWRU
Email: bst18@case.edu

Michael Tomasini
Rockefeller University
Email: michael.tomasini@rockefeller.edu

Bruce Torbett
The Scripps Research Institute
Email: betorbet@scripps.edu

Udana Torian
NIAID
Email: utorian@niaid.nih.gov

Trang Tran
University of Florida
Email: trangtran@ufl.edu

Stephanie Tristram-Nagle
Carnegie Mellon University
Email: stn@cmu.edu

Allison Tsatsa
Harvard
Email: Allisontsatsa@g.harvard.edu

Lynda Tuberty
Rutgers University
Email: lyndatv@cabm.rutgers.edu

Steve Tuske
Rutgers University
Email: tuske@cabm.rutgers.edu

Mudit Tyagi
The George Washington University
Email: tmudit@email.gwu.edu

Pradeep Uchil
Yale University
Email: pradeep.uchil@yale.edu

Obiaara Ukah
University of Missouri
Email: obif29@mail.missouri.edu

Aline Umuhire Juru
Duke University
Email: aline.umuhire.juru@duke.edu

CJ Umunna
NIH/NCI
Email: cj.umunnakwe@nih.gov

Emiko Urano
NCI
Email: uranoe@mail.nih.gov

S. "Vaithee" Vaitheeswaran
Schrodinger Inc.
Email: vaithee@schrodinger.com

Wilfred van der Donk
Howard Hughes Medical Institute and the University
Email: vddonk@illinois.edu

Greg Van Duyne
University of Pennsylvania School of Medicine
Email: vanduyne@mail.med.upenn.edu

Rachel Van Duyne
NIH/NCI
Email: rachel.vanduyne@nih.gov

Janani Varadarajan
Vanderbilt University Medical Center
Email: jan.varadarajan@vanderbilt.edu

Guelaguetza Vazquez-Meves
Howard University
Email: guelaguetza.vazquez@Howard.edu

Gregory Voth
University of Chicago
Email: gavoth@uchicago.edu

JÃ¶rg Votteler
University of Utah
Email: joerg.votteler@biochem.utah.edu

Jonathan Wagner
University of Virginia
Email: jonw7782@gmail.com

Kevin Wang
Yale University
Email: kevinwang2013@gmail.com

Mingzhang Wang
University of Delaware
Email: wmzmaple@udel.edu

Octavia Weatherspoon
NIGMS
Email: octavia.weatherspoon@nih.gov

Yi Wen
Cornell University
Email: wy31912@gmail.com

Troy Whitfield
University of Massachusetts Medical School
Email: Troy.Whitfield@umassmed.edu

Carl Wild
DFH Pharma, Inc.
Email: cwild@aol.com

James Williamson
The Scripps Research Institute
Email: jrwill@scripps.edu

Li Wu
Ohio State University
Email: wu.840@osu.edu

Marie Teng-Pei Wu
Harvard University
Email: marietengpeiwu@g.harvard.edu

Ying Wu
University of Pittsburgh
Email: yiw60@pitt.edu

Zhaoyong Xi
University of Pittsburgh
Email: zhx23@pitt.edu

Tsan Xiao
Case Western Reserve University
Email: tsx@case.edu

Guangai Xue
NCI
Email: xueg@mail.nih.gov

Mark Yeager
University of Virginia School of Medicine
Email: yeager@virginia.edu

Whitney Yin
University of Texas Medical Branch
Email: ywyin@gmail.com

Ashley York
The Rockefeller University
Email: ayork@adarc.org

Kaneil Zadrozny
University of Virginia
Email: kkz4n@virginia.edu

Jacqueto Zephyr
UMassmed
Email: jacqueto.zephyr@umassmed.edu

Jinwei Zhang
NIH
Email: jinwei.zhang@nih.gov

Kaiming Zhang
Baylor College of Medicine
Email: kaimingz@bcm.edu

Xue Zhi Zhao
NIH
Email: xuezhi.zhao@nih.gov

Xunhai Zheng
NIEHS
Email: zxh3210@gmail.com

Zhou Zhong
University of Pittsburgh
Email: zhouzhong@pitt.edu

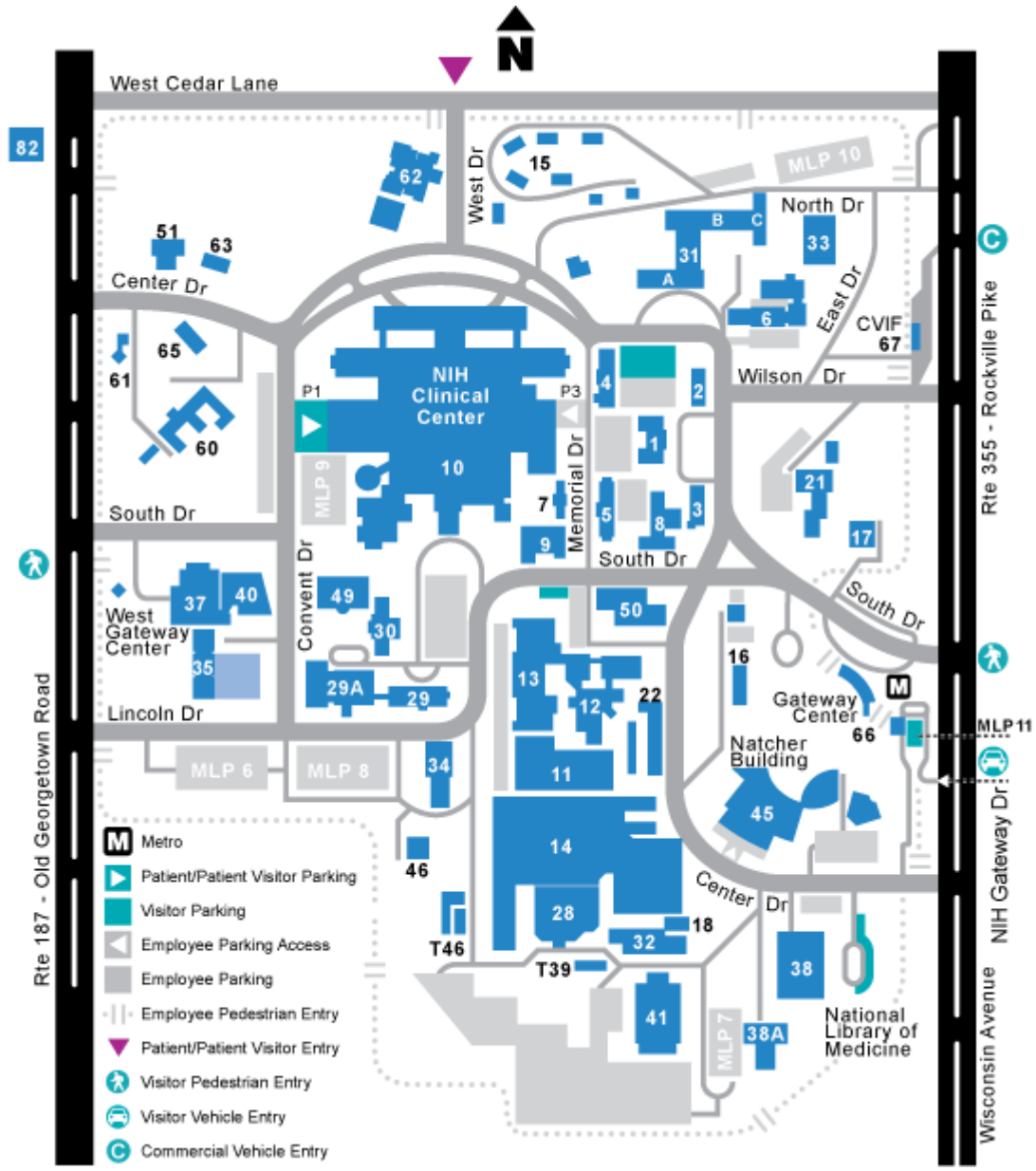
Shuntai Zhou
UNC Chapel Hill
Email: shuntaiz@email.unc.edu

Xiaohong Zhou
University of pittsburgh
Email: xiz134@pitt.edu

Jian Zhu
University of Rochester Medical Center
Email: Jian_Zhu@urmc.rochester.edu

Samantha Ziegler
Yale University
Email: samantha.ziegler@yale.edu

Map of NIH Campus



Some useful notes:

- The conference is located in Natcher (Building 45), southwest of the Gateway Center entrance to campus. The Medical Center Metro stop is next to the Gateway Center.
- Non-NIH employees must undergo inspection and receive temporary ID cards at the Gateway Center (see additional information on following pages).
- Parking garage MLP-11 is for non-NIH employees; NIH employees may park in the garage under Building 45 (requires car safety inspection next to Building 38A) or in other employee parking.
- Dining options on the NIH campus include Eurest Dining Services locations in Buildings 45, 1, 10, and 31, Maryland Business Enterprise Program for the Blind locations in Buildings 38A and 12B, and a concession stand in the Natcher lobby (http://does.ors.od.nih.gov/food/index.htm).
- An ATM is located in the Natcher lobby.

NIH Gateway Center Map

Main Visitor Entrance: NIH Gateway Drive
Gateway Center - Building 66 (for pedestrians entering campus)

Gateway Inspection Station - Building 66A (for vehicles entering campus)

• Monday – Friday: 5am – 10pm; Weekends and After Hours: Closed After hours: After 10pm on weekdays, all day weekends and holidays, pedestrians and visitors in vehicles should enter campus via the Commercial Vehicle Inspection Facility (CVIF) - Building 67 (on Rockville Pike between North Drive and Wilson Drive)
• After inspection, vehicles enter campus at Center Drive
• Roadway at Center Drive is for entering campus only; visitors exiting campus may exit from other open locations. To see a list of exits, please see the map.
• All vehicles and their contents will be inspected upon entering the campus.

Multi-Level Parking Garage 11 – MLP-11 (for parking outside of campus)

• Monday – Friday: 6am – 9pm (entrance) 6am – 11pm (exit) Weekends: Closed
• When MLP-11 is closed, visitors can park in lots on the NIH Campus
• Visitors parking in this garage should proceed to the Gateway Center (Bldg. 66) to get a visitor badge
• All visitors traveling in a vehicle are highly encouraged to park in MLP-11 as there is limited visitor parking on the main campus
• No vehicle inspection required to park in MLP-11
• Vehicles left in the MLP-11 parking garage after 11pm on weekdays or during any weekends are subject to ticketing and towing
• Cost: $2 per hour for the first three hours, $12 maximum for the entire day

Directions to NIH Gateway Drive from Rockville Pike/Wisconsin Avenue:
Southbound:

1. Continue on Rockville Pike past South Drive
2. Turn right at NIH Gateway Drive

Northbound – Option 1:

1. Continue on Rockville Pike past South Drive
2. Make a u-turn from the left turn lane at Wilson Drive
3. Continue southbound on Rockville Pike past South Drive
4. Turn right at NIH Gateway Drive

Northbound – Option 2:

1. Continue on Rockville Pike
2. Turn left at Battery Lane
3. Turn right on Old Georgetown Road
4. Turn right on Cedar Lane
5. Turn right on Rockville Pike
6. Continue southbound on Rockville Pike past South Drive
7. Turn right at NIH Gateway Drive

Northbound – Option 3:

1. Continue on Rockville Pike to South Drive
2. Make a u-turn from the left turn lane at South Drive
3. Continue southbound on Rockville Pike
4. Turn right at NIH Gateway Drive

Security Procedures for Entering the NIH Campus:
* All visitors and patients—**please be aware**: Federal law prohibits the following items on Federal property: firearms, explosives, archery equipment, dangerous weapons, knives with blades over 2 ½ inches, alcoholic beverages and open containers of alcohol.
* The NIH has implemented security measures to help ensure the safety of our patients, employees, guests and facilities. All visitors must enter through the NIH Gateway Center at Metro or the West Gateway Center. You will be asked to submit to a vehicle or personal inspection.
* Visitors over 15 years of age must provide a form of government-issued ID such as a driver's license or passport. Visitors under 16 years of age must be accompanied by an adult.

Vehicle Inspections – All vehicles and their contents will be inspected upon entering the campus. Additionally, all vehicles entering certain parking areas will be inspected, regardless of any prior inspection. Drivers will be required to present their driver's license and may be asked to open the trunk and hood. If you are physically unable to perform this function, please inform the inspector and they will assist you.
Vehicle inspection may consist of any combination of the following: Detection Dogs Teams (K-9), Electronic Detection Devices and Manual Inspection.
After inspection, you will be issued a vehicle inspection pass. It must be displayed on your vehicle's dashboard while you are on campus. The inspection pass is not a "parking permit." It only grants your vehicle access to enter the campus. You can only park in designated parking areas.

Personal Inspections – All visitors should be prepared to submit to a personal inspection prior to entering the campus. These inspections may be conducted with a handheld monitoring device, a metal detector and by visible inspection. Additionally, your personal belongings may be inspected and passed through an x-ray machine.

Visitor passes must be prominently displayed at all times while on the NIH campus.
To learn more about visitor and security issues at the NIH, visit:
http://www.nih.gov/about/visitor/index.htm.
For questions about campus access, please contact the ORS Information Line at orsinfo@mail.nih.gov or 301-594-6677, TTY - 301-435-1908.